Studies on the Application of Antibiotics as External Preservatives of Mango Cultivar of Fazli

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Philosophy in the Department of Chemistry

Khulna University of Engineering & Technology

Khulna-9203, Bangladesh

June 2015

Declaration

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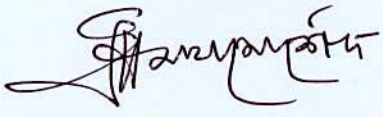

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Abstract

The application of various antibiotics at different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm) for the extension of storage life and quality of fazli mango was studied. The physical properties such as appearance, colour, flavor, taste and texture of all antibiotics treated mangoes were more attractive than those of control one. The storage life of treated mango was prolonged significantly as compared to that of control one. The weight loss control capacity of antibiotics treated mango at 20 ppm of tetracycline, amoxicillin 50 ppm, co-trimoxazole 20 and 30 ppm, cefradine 50 ppm, azithromycin 20 ppm was higher than that from control mango. The superior treatment tetracycline 20 ppm, co-trymoxazole 20 & 30 ppm and cefradin 50 ppm reduced the physiological loss in weight 15.79% to 33.62% with respect to control at 14th day. But at 15th day the treatments tetracycline 20 ppm, co-trymoxazole 20 ppm and cefradin 50 ppm reduced the physiological loss in weight 29.34% to 34.33% with respect to control mango. The nutritional qualities of mango were also affected remarkably after treatment with antibiotics. At the last edible stage chemical analysis of mango pulp from antibiotics treated mango at tetracycline 20 ppm, amoxicillin 50 ppm, co-trymoxazole 20 and 30 ppm, ciprofloxacin 20 ppm, cefradin 30 ppm, azithromycin 20 ppm, cefixime 20 and 30 ppm showed higher pH (5.25, 5.25, 6.20, 6.25, 6.15, 6.32, 5.65, 6.31 and 5.24), total soluble solids (TSS) (12.0%, 11.5%, 15.0%, 19.0%, 18.5%, 17.0%, 15.0%, 14.0% and 13.5%), total sugar (9.79, 6.59, 8.96, 9.77, 11.53, 9.99, 12.39, 9.80 and 10.32 g/100g), protein (0.79%, 1.03%, 0.38%, 0.54%, 0.49%, 0.39%, 0.46%, 0.60% and 0.50%), and iron (0.9344, 1.0529, 0.4602, 0.6204, 0.4010, 0.7858, 1.2985, 0.6909 and 0.7572 mg/100g) in comparison to control mango (pH = 5.19, TSS = 10%, total sugar = 10.9 g/100g, protein = 0.57% and iron = 0.7218 mg/100g). In comparison to control mango it is evident that the antibiotic treated mangoes might be in superior quality as it contains higher vitamin A, vitamin C, total soluble solids, total sugar, iron (Fe) and pH than those of control one.

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CHAPTER I

Introduction

1.1 General

The mango (*Mangifera indicia* L.) is the principal cash fruit crop of Rajshahi region [1] and it is one of the most important and valuable fruit of Bangladesh. It is also certainly one of the highly delicious and esteemed fruit of the world. Mango is a luscious and nutritious fruit and an excellent source of beta-carotene (pro-vitamin A), essential minerals, vitamin C, carbohydrate and energy in human nutrition [2]. Fresh mango fruit is considered as a "king of fruit" in Bangladesh and is appreciated as the choicest of indigenous fruits by millions of people [3]. Mangoes are still judged as luxurious and expensive items of the markets of many industrialized countries. It is extensively cultivated in Bangladesh, India, Pakistan, Philippines, Thailand, Sri Lanka, Malaysia, Israel, Africa, some parts of Australia and America. Mango is generally produced once in a year while many of commercial varieties are biennial in bearer. In our country, this fruits are obtained from the month of April-May to July-August.

1.2 Origin

The mango belongs to the family *Anacardiaceae.* It has been cultivated for more than 4000 years as described by De Candolle [4]. According to him originated in South Asia or Malayan Archipelago. Popnoe [5] mentioned that it probably originated in Eastern India, Assam and Burma or further in the Malayan region. Mukherjee [6] reported that the genus *Mangifera* originated in Burma, Siam, Indo-china and the Malayan Peninsula; but the mango itself had its origin in which includes the area what is now Bangladesh. The places of origin of mango are shown in figure 1.1. The wild mangoes particularly, *M. sylvatica Roxby*,

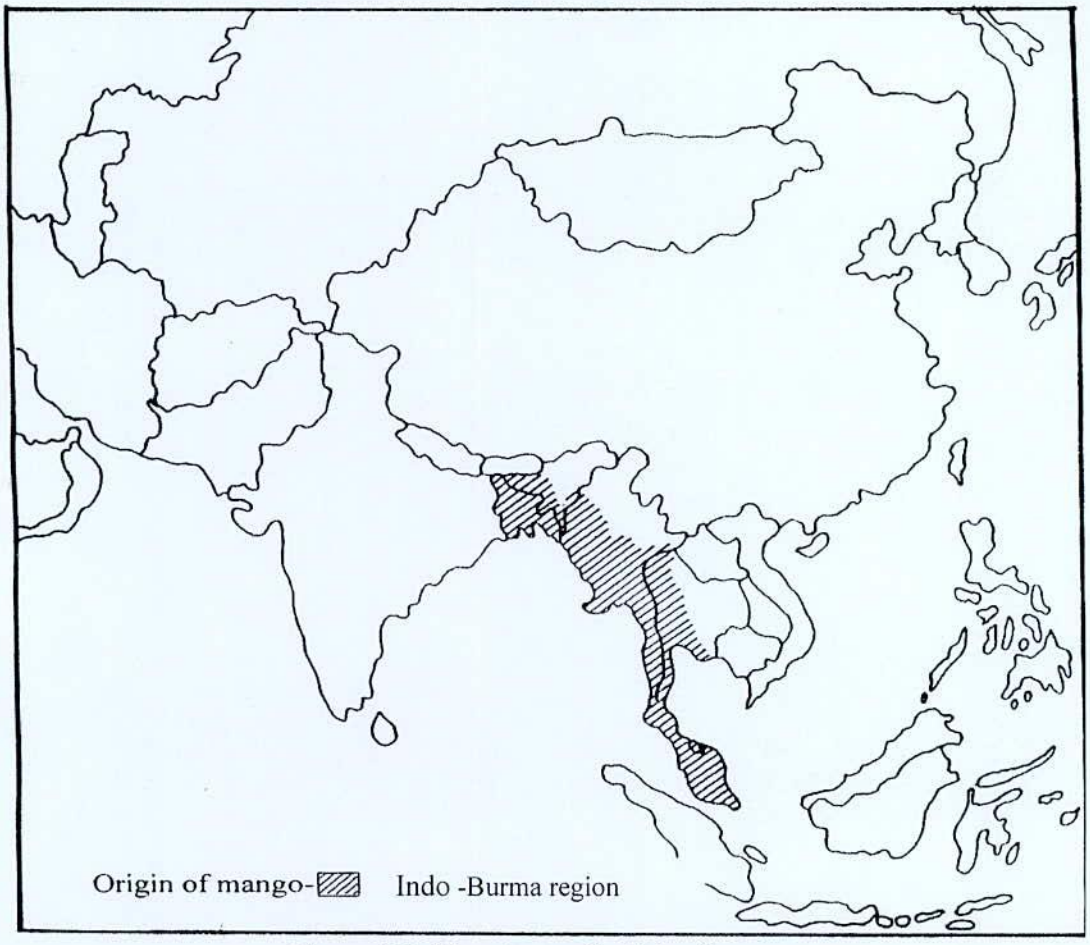


Figure 1.1: The places of origin of mango.

are still found in the Chittagong Hill Tracts of Bangladesh [7]. Vavilov [8] had also the same opinion that the mango was originated in the Indo-Burma region. Bangladesh is proud to be the home of mango, one of the most important fruits of the world.

1.3 Species varieties

Vegetative propagation which started 400 years back in India has helped to perpetuate outstanding chance seedlings. However, names of mango varieties remained ever confusing. The same variety has assumed different names in different places. This is further aggravated due to the fact that a variety can't be identified by vegetative characters alone. A variety introduced from one region to another may not behave the same way. It is reported that Langrage and Dusehri of Uttar Pradesh of India grown in Madras of the same country did not show resemblance to the original parent in respect of flavour, size and other characteristics [9]. On the other hand, if there is a search for high

yielding, disease resistant, regular bearing varieties all desirable characters may not be found in one variety. However, all desirable characters may be combined in a variety through a systematic hybridization programme. So there is need for characterization of existing varieties.

Twelfth International Horticultural congress held at Berlin in 1938 recognized the importance of description and classification of varieties as a fundamental aspect of fruit research. It was affirmed at the Indian Horticultural Workers Conference held in New Delhi in 1947. Watt [10] was the earliest in describing mango using scientific terminology. Subsequently Maries [11] described 500 varieties of Indian mango. Woodhouse [12] described 40 mango varieties of Bihar while Burns & Prayag [13] described 89 varieties of Bombay Presidency. Popnoe [14] described 300 varieties of mango of all parts of the world. Sturrock and Wolfe [15] described 38 mango varieties of florida based on fruit characters only. All the workers did not include vegetative characters of varieties in their description. However, Mukherjee [16] who described 72 varieties of Bengal, Bihar and Uttar Pradesh while Naik and Gangolly [17] who described 335 varieties of South India used vegetative characters as well.

The cultivated mangoes in different regions of the world belong to different species but the mango varieties of Bangladesh belong to *Mangifera indica* L. The mango varieties of Philippines, Thailand and Indonesia are poly-embryonic. However, the mango varieties of Bangladesh are mono-embryonic and cross pollinated. The number of quality mango varieties cultivated in Bangladesh are not many. It is estimated to be around 250. However, there are many more varieties which are not yet commercially important but maintained at family level [7]. The four main groups of mango varieties are the Indian, Floridian, Indonesian and Philippine [18].

Many varieties of mango are now available in Bangladesh. Of which important cultivars are listed below.

Table 1.1: Varieties of mango

1.	Fazli	2.	Aswina
3.	Langra	4.	Khirsapat
5.	Gopalbhog	6.	Mohonbhog
7.	Misribhog	8.	Kishanbhog
9.	Rajbhog	10.	Baishaki
11.	Himsagar	12.	Lakhanbhog
13.	Lata bombai	14.	Ranipasand
15.	Surjapuri	16.	Kuapahari
17.	Ilsapeti	18.	Misrikanta
19.	Dilsad	20.	Amrita bhog.

1.4 Nutritional and medicinal value of mango

Importance of mango in human diet is well recognized. In fact, the juicy pulp, attractive colour, excellent flavour, delicious taste and nutritional value of mango pulp readily command attention of the consumers. Our diet is very poor and lack in essential constituents like vitamins and minerals. More that 80% of the people of Bangladesh are suffering from severe malnutrition. Malnutrition may be due to deficiency in proteins, vitamins and minerals. Mangoes are excellent source of vitamin like pro-vitamin A, vitamin B_1, vitamin B_2, folic acid and vitamin C, which help in the maintenance of proper health and resistance to diseases. It also provides minerals, such as iron (Fe), calcium (Ca) and phosphorus (P), the deficiency of which may lead to disturbance in the metabolism and can cause several ailments.

In comparison with banana, papaya and jackfruit, which are generally considered to be above average in nutritional qualities and on the basis of nutrient content mango fruit

might be superior to banana, papaya and jackfruit [19]. The nutritional composition of the above four fruits are shown in table 1.2.

Table 1.2: A comparison on nutritional composition of four different types of fruits (100 g edible portion) [19]

Name of nutrient		Mango	Jackfruit	Banana	Papaya
Water (%)		88.6	78.0	62	88.4
Food energy (Cal/100g)		90.0	48.0	109	42
Total carbohydrate (%)		20	9.9	25	8.3
Protein (%)		1.0	1.8	1.2	1.9
Lipid (%)		0.7	0.1	0.8	0.2
Fibre (%)		0.7	0.2	0.4	0.8
Ash (%)		0.6	0.3	0.5	0.2
Calcium (mg/100g)		16	26	13	19
Phosphorus (mg/100g)		20	30	19	10
Iron (mg/100g)		1.3	0.5	0.9	0.5
Vitamin A (μg/100g)		8300	4700	500	8100
Vitamin B_2 (μg/100g)		0.07	0.15	0.05	0.03
Vitamin C (mg/100g)		90	21	24	42

Mango also supplies carbohydrates, proteins and fats. At initial stages of fruit development no systematic trend was observed in the sugar content, but toward the end of maturity, both reducing and non-reducing sugars were found to be increasing [20]. Leley observed an increase from 1 to 13% in starch content in Alphonso mango during development [21]. Mann recorded a gradual decrease in acidity until harvest in Dashehari mangoes [20]. Pathak and Sarada reported that lipid content in pulp of five mango varieties ranged from 0.80-1.36% at harvest [22] while pulp chlorophyll became negligible as the fruit approached maturity [23]. The total carotenoides and β-carotene remained very low initially and increased gradually as the fruits approached maturity and ripening but ascorbic acid gradually decreased as the fruits approached maturity [24]. Mango fruit contains 0.5-1% proteins on a fresh weight basis [25]. Tandon and Kalra [23] reported a decrease in the soluble protein content up to 44 days after fruit set, which increased again until 96 days.

Carotenoids are mainly responsible for the color of ripe mangoes. The composition of the carotenoides in Badami (Alphonso) mangoes were characterized by Subrayan and Cama [26] at three stages of maturity-unripe, partially ripe and fully ripe stages they found 14, 15 and 17 different carotenoids respectively. In fully ripe mangoes β-carotene constituted 50-64% of the total, with phylofluence (11.7%). quroxanthin (11.4%) cis-violananthin (7.08%) and phyloene (6.32%) comprising the other major carotenoids. The red blush in haden mango is attributed to the presence of the anthocyanin and peonidin-3-galactoside [27].

Many medicinal properties are also ascribed to mango. Dried flowers have curative properties for treating diarrhoea and chronic dysentery. The smoke burning leaves is believed to be efficacious against hiccough and several throat troubles. Bark yields mangiferine and tannin which are useful against diphtheria and rheumatism. The kernel is being given as medicine to persons suffering from asthma and diarrhoea. Barked and sugared pulp of unripe fruit is being considered very useful for cholera and plague patients.

The bark is a source of resins and gum. The gum of the tree and the resinous substance excluded from the stem end of the harvested fruits are mixed with lime juice and given in case of coetaneous affections and scabies.

1.5 In which way mangoes are infected

Mangoes are infected *by Xanthomonas campestris* bacteria [28].

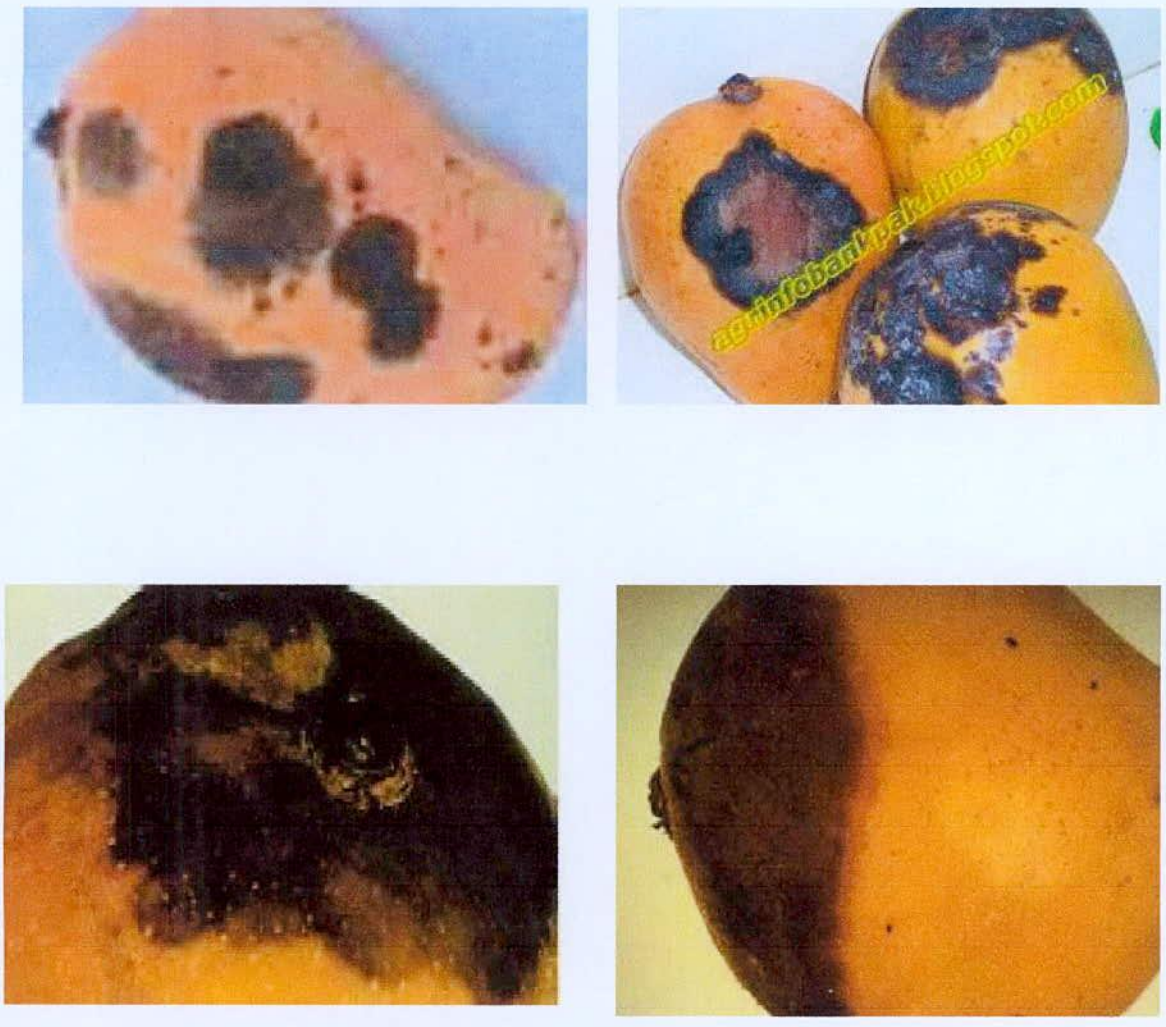


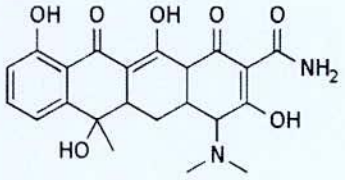
Figure 2.1: Some infected mangoes [29].

1.6 Antibiotics

Antibiotics are secondary metabolites produced by microorganisms that inhibit or kill a wide spectrum of other microorganisms.

1.6.1 Tetracycline

The first member of the group to be discovered is chlortetracycline (aureomycin) in the late 1940s by Benjamin Minge Duggar, a scientist employed by American Cyanamid - Lederle Laboratories, under the leadership of Yellapragada Subbarow, who derived the substance from a golden-colored, fungus-like, soil-dwelling bacterium named *Streptomyces aureofaciens* [30]. Oxytetracycline (terramycine) was discovered shortly afterwards by *AC Finlay et al.*; it came from a similar soil bacterium named *Streptomyces rimosus* . Robert Burns Woodward determined the structure of oxytetracycline enabling Lloyd H. Conover to produce tetracycline successfully itself as a synthetic product.

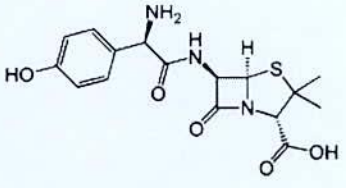


Structure 1.1: Tetracycline.

Tetracyclines are a group of broad-spectrum antibiotics whose general usefulness has been reduced with the onset of antibiotic resistance. Despite this, they remain the treatment of choice for some specific indications.

1.6.2 Amoxicillin

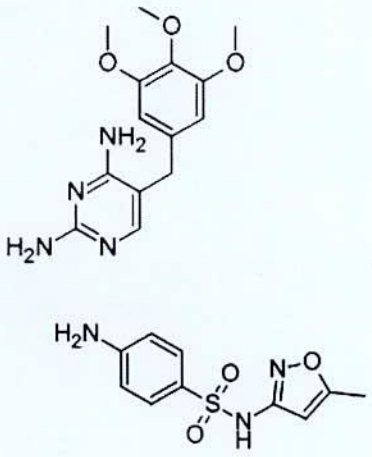
Amoxicillin was one of several semisynthetic derivatives of 6-aminopenicillanic acid (6-APA) developed at Beecham, England in the 1960s. It became available in 1972, and was the second aminopenicillin to reach the market (after ampicillin in 1961) [31, 32].



Structure 1.2: Amoxicillin.

Amoxicillin belongs to a group of drugs called the penicillins which originate from a form of fungi called Penicillium fungi. Penicillins are antibiotic drugs, meaning that they are used to treat infections caused by bacteria and to eliminate the bacteria themselves. Amoxicillin fights bacteria by preventing them from forming cell walls and stopping them from growing. This kills the bacteria and eventually heals the infection .

1.6.3 Co-trimoxazole



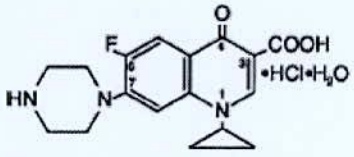
Structure 1.3: Trimethoprim (top) and sulfamethoxazole (bottom).

Trimethoprim/sulfamethoxazole (TMP/SMX) or co-trimoxazole (BAN) is an antibiotic used to treat a variety of bacterial, fungal, and protozoal infections. It consists of one part trimethoprim to five parts sulfamethoxazole. The drug has been marketed worldwide in generic preparations and under multiple brand names, including Septra (GlaxoSmithKline plc) and Bactrim (Hoffmann-La Roche). Co-trimoxazole is generally considered bactericidal, although its components are individually bacteriostatic [33,34]. It is an antifolate drug and functions by inhibiting both de novo folate biosynthesis and metabolism.

1.6.4 Ciprofloxacin

Ciprofloxacin (INN) is an antibiotic that can treat a number of bacterial infections. It is a second-generation fluoroquinolone [35-37]. Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including gram-negative (*Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*,

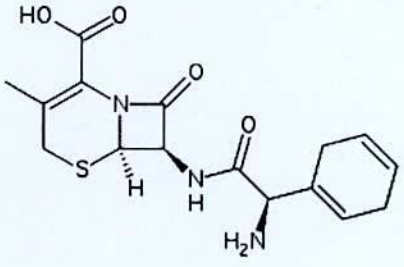
Proteus mirabilis, and *Pseudomonas aeruginosa*), and gram-positive (methicillin-sensitive, but not methicillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Streptococcus pyogenes*) bacterial pathogens. Ciprofloxacin and other fluoroquinolones are valued for this broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations [38].



Structure 1.4: Ciprofloxacin.

1.6.5 Cefradine

Cefradine (INN) (formerly cephradine BAN) is a first generation cephalosporin antibiotic [39].

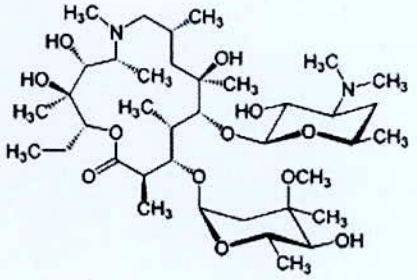


Structure 1.5: Cefradine.

1.6.6 Azithromycin

Azithromycin is an antibiotic useful for the treatment of bacterial infections. It is an azalide, a subclass of macrolide antibiotic. It is derived from erythromycin, with a methyl-substituted nitrogen atom incorporated into the lactone ring, thus making the lactone ring 15-membered. Azithromycin is somewhat more potent against certain bacterial species than erythromycin, but its widespread popularity arises primarily from its slow elimination from the body, which allows many infections to be treated with 3-5

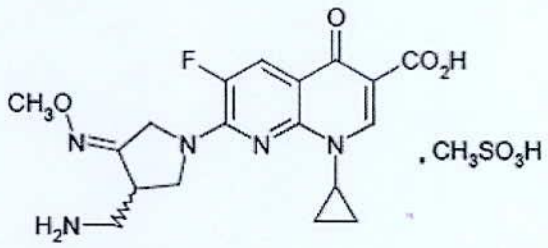
days of once-daily administration, compared to 3-4 times a day for up to two weeks for erythromycin [40, 41].



Structure 1.6: Azithromycin.

1.6.7 Gemifloxacin

Gemifloxacin, a compound related to the fluoroquinolone class of antibiotics, is available as the mesylate salt in the sesquihydrate form. Chemically, gemifloxacin is (R, S)-7[(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo1, 8-naphthyridine-3-carboxylic acid . Gemifloxacin is considered freely soluble at neutral pH (350 μg/mL at 37°C, pH 7.0).



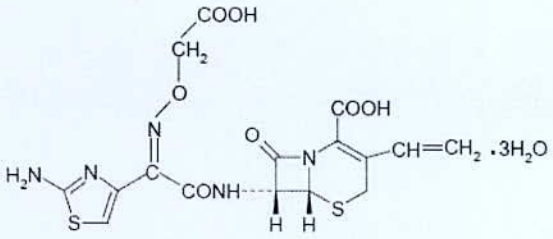
Structure 1.7: Gemifloxacin.

Gemifloxacin, the inactive ingredients are crospovidone, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, povidone, and titanium dioxide [42, 43].

1.6.8 Cefixime

Cefixime is an antibiotic useful for the treatment of a number of bacterial infections. It is a third generation cephalosporin developed by Wyeth Pharmaceuticals. It

is on the World Health Organization's list of essential medicines, a list of the most important medication needed in a basic health system [42, 44].



Structure 1.8: Cefixime.

1.7 Aim of the present study

Mango, a mostly available seasonal fruit in Bangladesh is liked by millions of people due to its excellent flavor and taste. Approximately 30-50% fruits go waste during postharvest handling, storage and ripening [45]. Among the fruits mango manifested high postharvest losses because of its high perishability and climacteric pattern of respiration. The marketability of this perishable fruit is closely linked with the development of suitable technology which reduces the loss of storage life.

The postharvest life of any fruit consists of ripening and senescence. After harvest, fruits undergo many physiological and biochemical changes during storage. Apart from those changes, microbial decay also contributes to postharvest losses during ripening and storage. The storage life of a fruit could be prolonged significantly through slowing down the process leading to ripening, and controlling the microbial decay.

The physico-chemical changes during ripening and storage need to be studied extensively to develop more effective technique of prolonging economic storage life of mango. Nutritional and edible qualities of mango are affected by application of the post harvest treatments and also by harvesting the fruits at various stages of maturity.

Several authors [46-49] studied the postharvest losses and physico-chemical changes during ripening and storage of mango. But such studies are inadequate to explain the situation in our country.

Therefore, there is a need for detailed studies to develop a technology that will delay the ripening process of the commercial varieties of fazli mango of Rajshahi zone. Studies are also needed to reduce post harvest losses and to extend shelf life of fazli mango. The present study was therefore undertaken with the following objectives.

1. to select and search out the new preservatives (antibiotics) of mango
2. to increase the shelf life of fazli mango
3. to retain the quality and characteristics of mango
4. to select the suitable doses of different antibiotics for mango cultivar of fazli.

CHAPTER II

Literature Review

2.1 Effects of preservatives on the shelf life and post harvest losses of mango

The storage life of any fruit consists of ripening and senescence. After harvest, fruits undergo many physiological and biochemical changes during storage. The storage life of fruits could be prolonged significantly through slowing down the process leading to ripening and controlling the microbial decay [50, 51].

Approximately 30-50 % fruits go waste during post harvest handling, storage, and ripening [45, 52]. Among the fruits mango manifested highest post harvest losses because of its high perishability and climacteric pattern of this fruit is closely linked with the development of suitable technology which reduces the losses at different stages of harvesting, packaging, and storage. Quality mangoes are produced in north-western part of Bangladesh, of which about 35-38% post harvest losses are caused due to inefficient handling during its transportation, storage, and marketing [53]. The effects of plant hormones on the shelf life and post harvest losses were also reported [54].

2.2 Effects of preservatives on the improvement of quality of mango

Mango is now recognized as one of the best fruit of all indigenous fruits due to its excellent flavor, attractive fragrance, and beautiful shades of colour, delicious taste, and high nutritional value (i.e. quality parameters). It is also a luscious and nutritious fruit and chief source of beta-carotene (pro-vitamin A), ascorbic acid (vitamin C), essential minerals (basically-calcium, phosphorus and iron), carbohydrate, and energy in human nutrition [1, 55-57]. Quality fruits are important ingredients of human diet and also useful for processing, quality mangoes are produced in north-western part of Bangladesh, of which about 35-38% post harvest losses are caused due to inefficient handling during its transportation, storage and marketing [53]. The qualities of preserved mango were also developed using plant hormones [54].

CHAPTER III

Methodology

3.1 Treatments and determination of shelf life

Freshly harvested uniformly ripe mango cultivar of fazli is collected from the experimental mango research garden of Kansat, Chapainababganj, during July and August 2014. During the period of study the ambient temperature and relative humidity in the laboratory ranged between 30-35°C and 75-80% respectively. Only sound and firm ripe 690 mangoes that are averagely uniform size, shape, and colour were used in this experiment. The mangoes were divided in 46 lots, containing 15 mangoes in each lot and the treatments were made by eight different antibiotics. Tetracyclin, amoxicillin, co-trimoxazole, ciprofloxacin, cefradine, azithromycin, cefixime, and gemifloxacin. Tetracycline and amoxicillin in ten concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm), co-trimoxazole, ciprofloxacin and cefradine in five concentrations (10, 20, 30, 40 and 50 ppm), azithromycin, cefixime and gemifloxacin in three concentrations (10, 20 and 30 ppm) were used in this experiment. All the antibiotics were collected from local marcket of Square Pharmaceuticals Ltd, Bangladesh and desolved in water to make solution for the treatments of mango. There were 2 lots of control mango. So there were altogether 46 treatments including the control. The lots of mangoes under experiments were marked carefully. The lots of mangoes were dipped for 5 minutes in eight different antibiotic's solutions (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm). The control lots were marked and kept at room temperature (30-35°C) in identical condition.

For determining the physiological weight losses, the initial weight was recorded just before the treatment. Subsequently, their weights were recorded daily and the loss in weight was expressed as the percentage over the initial weight. To determine the shelf life, all the lots were observed every day at 5 pm for all treatments.

3.2 Physical properties and chemical analysis

This study includes detailed nutrient analysis of commercially important cultivar of fazli mango. During post harvest period of control and antibiotic treated mango. The experimental designs were as described in article 3.1. Freshly harvested mangoes for control and antibiotic treatments were collected from mango garden of Kansat,

Chapainababganj, Bangladesh. All the reagents used in the analysis were of analytical grade (MERCK, GERMANY).

3.2.1 Determination of pH

Extraction of mango juice: About 1-2 g of mango pulp was taken in a mortar. The pulp was crushed thoroughly in a mortar with pestle and homogenized well, and then filtered through two layers of cotton cloth. The filtrate was then centrifuged for 5 min. at 5000 rpm and the clear supernatant was collected.

Standard buffer solution

pH 7.0 or 4.0 buffer tablet (BDH Chemicals Ltd. Poole England) was dissolved in distilled water and made up to the mark of 100 mL with distilled water.

Procedure

The electrode assembly of the pH meter was dipped into the standard buffer solution of pH 7.0 taken in a clear and dry beaker. The temperature correction knob was set to 28°C and the fine adjustment was made by asymmetry potentially knob to pH 7.0. After wash the electrode assembly was then dipped into a solution of standard pH 4.0 and adjusted to the required pH by fine asymmetry potentially knob. The electrode assembly was raised, washed with distilled water, rinsed off with juice of the cultivars and dipped into the juice of the mango pulp. The pH of the juice was noted.

3.2.2 Estimation of total titrable acidity

The total titrable acidity of mango pulp was determined by titrimetric method [58].

Reagents

Stander NaOH solution (0.1N).

1 % Phenolphthalein solution.

Extraction of mango pulp juice: The mango pulp juice was extracted by the procedure same as described previously.

Procedure

Mango pulp juice was taken in a conical flask. Two to three drops of phenolphthalein indicator was added and mixed thoroughly. It was then titrated immediately with 0.1N NaOH solution from a burette till a permanent pink colour was appeared. The volume of NaOH solution required for titration was noted. The percentage of total titrable acidity present in the mango pulp was determined using the formula givenbelow.

Calculation

Amount of acidity in the mango pulp (g per 100 g of mango pulp)

$$= \frac{\text{Volume of alkali needed for titration} \times \text{Strength of alkali} \times \text{Eq. wt. of acid} \times 100}{\text{Weight of mango pulp} \times 1000}$$

3.2.3 Determination of moisture

Moisture content was determined by the conventional procedure [59].

Materials

a) Porcelain crucible.
b) Electrical balance
c) Oven.
d) Desiccator

Procedure

About 1-2 g of mango pulp was weighed in a porcelain crucible (which was previously cleaned and heated to about 100°C. cooled and weighed). The crucible with the sample was heated in an electrical oven for about six hours at 70°C. It was then cooled in a desiccator and weighed again until the weight became constant.

Calculation

Amount of moisture in the mango pulp (g per 100 g of mango pulp)

$$= \frac{\text{Weight of mositure obtained}}{\text{Weight of mango pulp}} \times 100$$

3.2.4 Determination of total soluble solids (TSS)

The total soluble solids (TSS) content of mango pulp was directly determined from the percentage scale (0-90 %) of Kyowa hand refract meter [58]. A drop of juice squeezed from control and antibiotics treaded mango pulp was placed on the prism of refract meter and percent of total soluble solids was obtained from direct reading.

3.2.5 Determination of total sugar

Total sugar content of mango pulp was determined calorimetrically by the enthrone method as described in Laboratory Manual in Biochemistry [60]. Enthrone reagent: The enthrone reagent was prepared by dissolving 2 g of enthrone in 1 liter of concentrated H_2SO_4.

a) Standard glucose solution: A standard solution of glucose was prepared by dissolving 10 g of glucose in 100 mL of distilled water.

Extraction of sugar from mango pulp :

Extraction of sugar from mango pulp was done following the method described by Loomis and Shull [61]. Four to six g of mango pulp were plunged into boiling ethyl alcohol and allowed to boil for 5-10 min (5 to 10 mL of alcohol was used for every g of mango pulp). The extract was cooled and crushed thoroughly in a mortar with a pestle. Then the extract was filtered through two layers of cotton cloth and re-extracted the ground tissue for three min in hot 80% alcohol, using 2 to 3 mL of alcohol for every g of sample. This second extraction ensured complete removal of alcohol soluble substances. The extract was cooled and passed through cotton cloth. Both the extracts were filtered through Whitman No-41 filter paper.

The volume of the extract was evaporated to about 1/4th the volume over a steam bath and cooled. This reduced volume of the extract was then transferred to a 100 mL volumetric flask and made up to the mark with distilled water. Then 1 mL of the diluted solution was taken into another 100 mL volumetric flask and made up to the mark with distilled water (working standard).

Procedure

Aliquot of 1 mL of the extract was pipette into test tube and 4 mL of the enthrone reagent was added to each of this solution and mixed well. Glass marbles were placed on the top of each tube to prevent loss of water by evaporation. The test tubes were heated for 10 min in a boiling water bath and then cooled. A reagent blank was prepared by taking 1 mL of water and 4 mL of enthrone reagent in a tube and treated similarly. The absorbance of the blue green solution was measured at 625 nm using the blank.

The amount of total sugar content in mango pulp was calculated by constructing a calibration curve using glucose as standard.

A standard curve of glucose was prepared by taking 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mL of standard glucose solution in different test tubes containing 0.0, 10 μg, 20 μg, 40 μg, 60 μg, 80 μg and 100 μg of glucose respectively and made the volume up to 1.0 mL with distilled water. Then 4 mL of enthrone reagent was added to each test tube and mixed well. All these solutions were treated similarly as described above. The absorbance was measured at 625 nm using the blank containing 1 mL of water and 4 mL of enthrone reagent. The amount of total sugar was calculated from the standard curve of glucose (Figure 3.1). Finally, the percentage of total sugar present in the mango pulp was determined using the formula given below.

Calculation

Amount of total sugar in the mango pulp (g per 100 g of mango pulp)

$$= \frac{\text{Amount of total sugar obtained}}{\text{Weight of mango pulp}} \times 100.$$

3.2.6 Determination of reducing sugar

Reducing sugar content of mango pulp was determined by dinitrosalicylic acid method [62].

Reagents

a) Dinitrosalicylic acid (DNS) reagent: simultaneously 1 g of DNS, 200 mg of crystalline phenol and 50 mg of sodium sulphite were placed in a beaker and mixed with 100 mL of 1% NaOH solution by stirring. If it is need to store then sodium sulphite must be added just before use.

b) 40% solution of Rochelle salt.

Extraction of reducing sugar from mango pulp

Reducing sugar extract from mango pulp was done by the procedure as described earlier.

Procedure

Aliquot of 3 mL of the extract was pipette into test tubes and 3 mL of DNS reagent was added to each of this solution and mixed well. The test tubes were heated for 5 min in a boiling water bath. After the color has developed 1 mL of 40% Rochelle salt was added when the contents of the tubes were still warm. The test tubes were then cooled under a running tap water. A reagent blank was prepared by taking 3 mL of water and 3 mL of DNS reagent in a tube and treated similarly. The absorbance of the solution was measured at 575 nm in a colorimeter. The amount of reducing sugar content in mango pulp was calculated by constructing a calibration curve using glucose as standard (Figure 3.2).

Calculation

Amount of reducing sugar in mango pulp (g per 100 g of mango pulp)

$$= \frac{\text{Amount of reducng sugar obtained}}{\text{Weight of mango pulp}} \times 100$$

3.2.7 Determination of non-reducing sugar

Non-reducing sugar was calculated from the following formula.

Non-reducing sugar = (% total sugar - % reducing sugar)

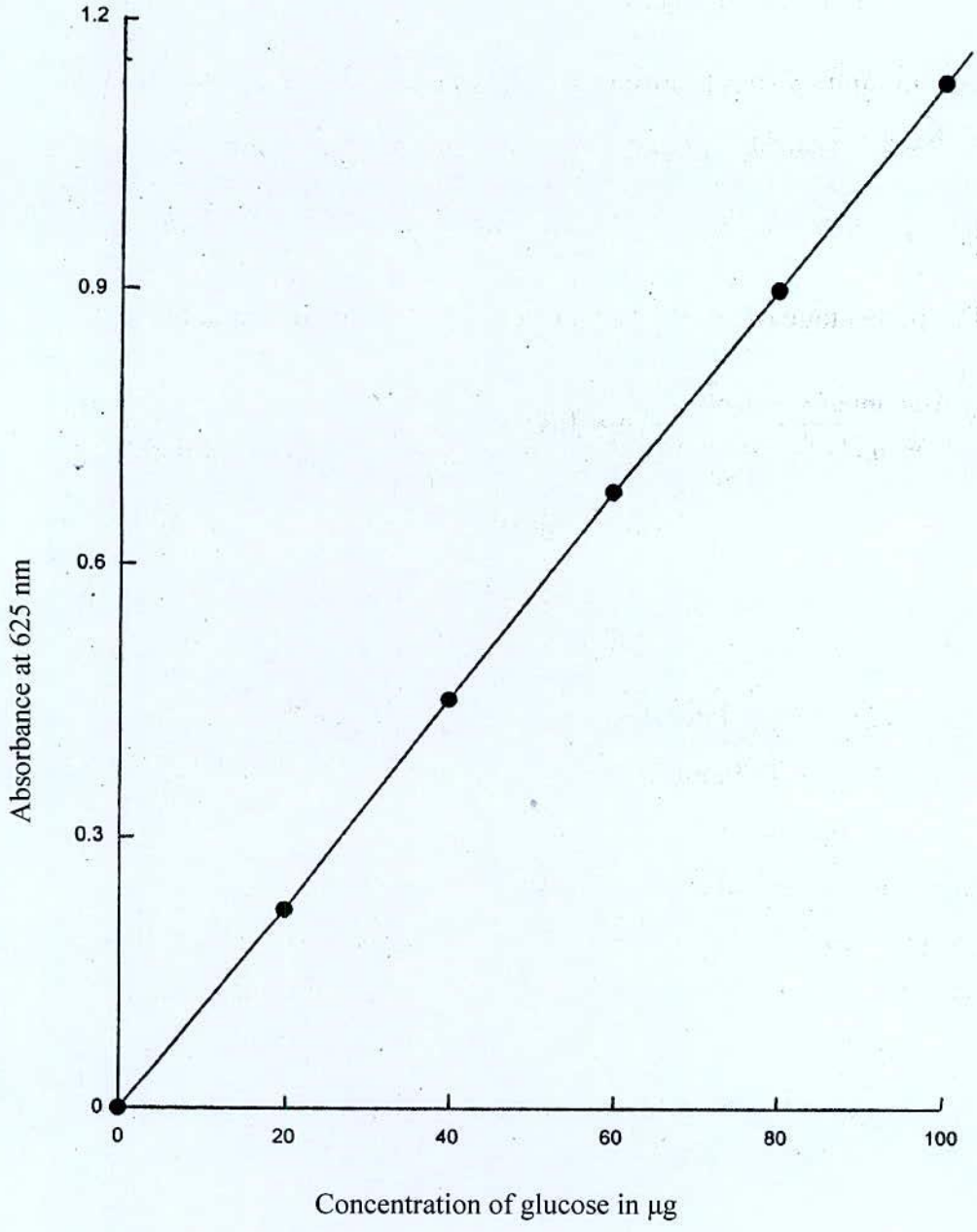


Figure 3.1: Standard curve of glucose for estimation of total sugar.

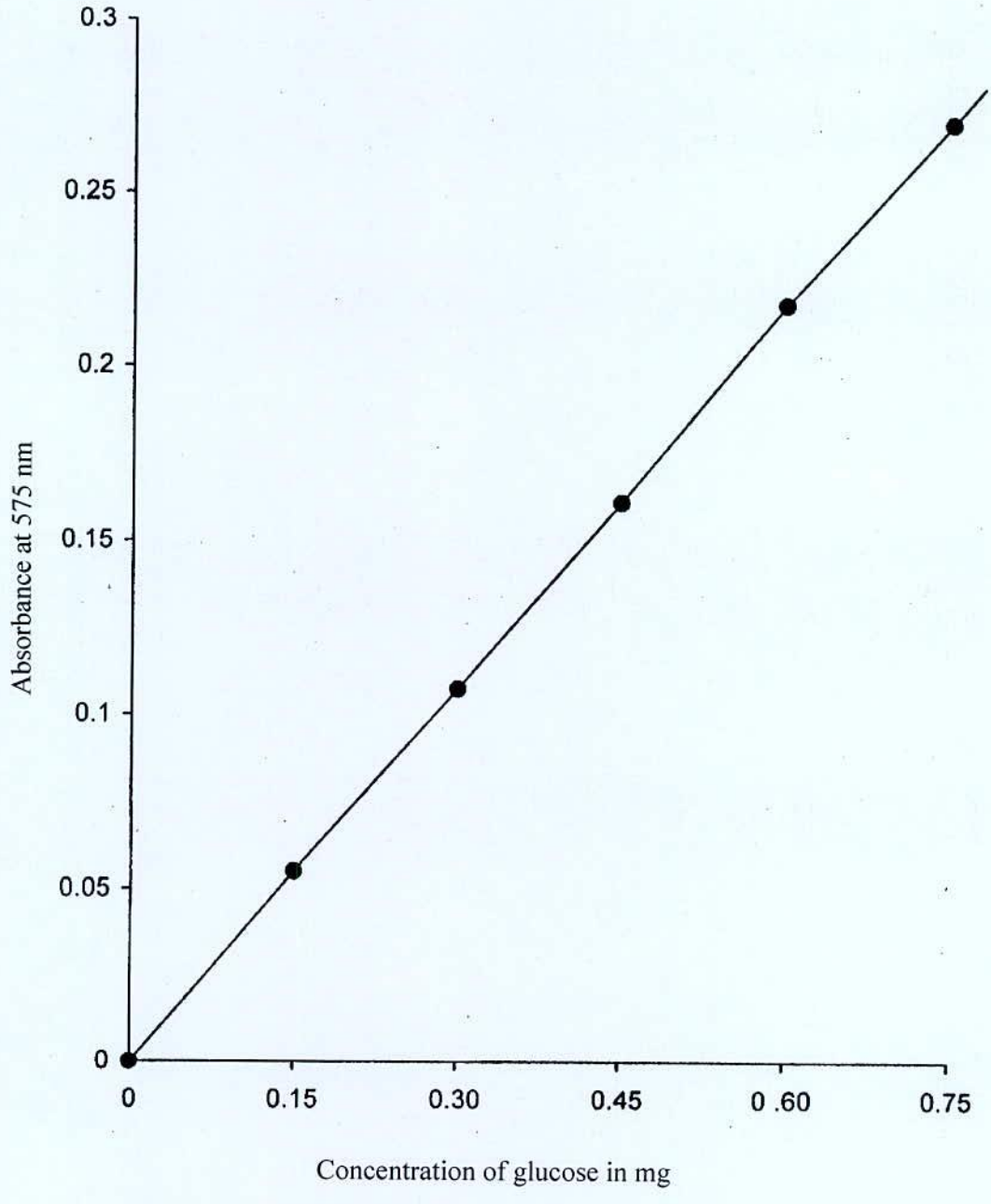


Figure 3.2: Standard curve of glucose for estimation of reducing sugar.

3.2.8 Estimation of ascorbic acid (vitamin C)

Ascorbic acid (vitamin C) of mango pulp was determined by the titrimetric method [63].

Reagents

a) Dye solution: 200 mg of 2,6-dichlorophenol indophenols and 210 mg of sodium bicarbonate were dissolved in distilled water and made up to 1000 mL. The solution was then filtered.
b) 3% Meta phosphoric acid reagent: 3 g of Meta phosphoric acid was dissolved in 80 mL of acetic acid and made up to 100 mL with distilled water.
c) Standard ascorbic acid solution (0.1 mg/mL): 10 mg of pure ascorbic acid was dissolved in 3% Meta phosphoric acid and made up to 100 mL with 3% Meta phosphoric acid.

Procedure

10 mL of standard ascorbic acid solution was taken in a conical flask and titrated it with the dye solution.

Four to six g of mango pulp were cut into small pieces and homogenized well with 3% meta phosphoric acid (approximately 20 mL) and filtered it through double layer of cotton cloth. The filtrate was centrifuged at 3,000 rpm for 10 min and the clear supernatant was titrated with 2, 6-dichlorophenol indophenols solution. The amount of ascorbic acid present in the mango pulp was determined by comparing with the titration result of standard ascorbic acid solution.

Calculation

Amount of ascorbic acid in mango pulp (mg per 100 g of mango pulp)

$$= \frac{\text{Amount of ascorbic acid obtained}}{\text{Weight of mango pulp}} \times 100$$

3.2.9 Estimation of β-carotene

β-carotene of mango pulp was determined according to the procedure reported in the Methods of Vitamin Assay [64] and Methods of Biochemical Analysis [65].

Reagent

a) Ammonium sulphate.
b) Acetone.
c) Petroleum ether (40°-60°C)
d) Potassium hydroxyde solution (5.6%)
e) n-Hexane
f) Activated alumina (BDH Chemicals Ltd).
g) Standard solution of β-carotene: A standard solution of β-carotene (BDH Chemicals Ltd.) was prepared by dissolving 50 mg of β-carotene in 100 mL of petroleum ether.

Column preparation

A column (40 × 2.5 cm) was prepared by using alumina as a packing material. 10% acetone in petroleum ether was used as eluant buffer.

Procedure

Five g of mango pulp and about four g of ammonium sulphate were taken in a mortar, and rubbed to an even paste with pestle. The extraction was carried out with acetone and small amount of hexane. Extraction was continued until the acetone extract became colorless. 10 mL of potassium hydroxide solution (5.6%) was added to the extract and it was kept in a dark place for half an hour. The mixture was then transferred to a separating funnel. 20 mL of petroleum ether, a few mL of hexane and 10 mL of water were added to the separating funnel and shacked gently. The ether layer was collected and the process was repeated until the petroleum ether layer became colorless. The concentrated extract (1-2 mL) was applied onto the top of the alumina column and eluted with 10% acetone in petroleum ether. The absorbance of the eluant was taken at 440 nm in a Coleman Junior 11 spectrophotometer.

Construction of standard curve of β-carotene

A standard curve was prepared by taking 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mL of standard solution in different test tubes containing 0.0 μg, 50 μg, 100 μg, 200 μg, 300 μg, 400 μg and 500 μg of β-carotene respectively and made the volume up to 5.0 mL with petroleum ether and mixed well. The absorbance of the solutions were taken at 440 nm in a Coleman Junior 11 spectrophotometer and a standard curve of β-carotene was prepared by plotting the data.

The amount of β-carotene present in the mango pulp was calculated by using standard curve (Figure 3.3).

Calculation

Amount of β-carotene in the mango pulp (μg per 100 g of mango pulp)

$$= \frac{\text{Amount of } \beta\text{ - carotene obtained}}{\text{Weght of mango pulp}} \times 100$$

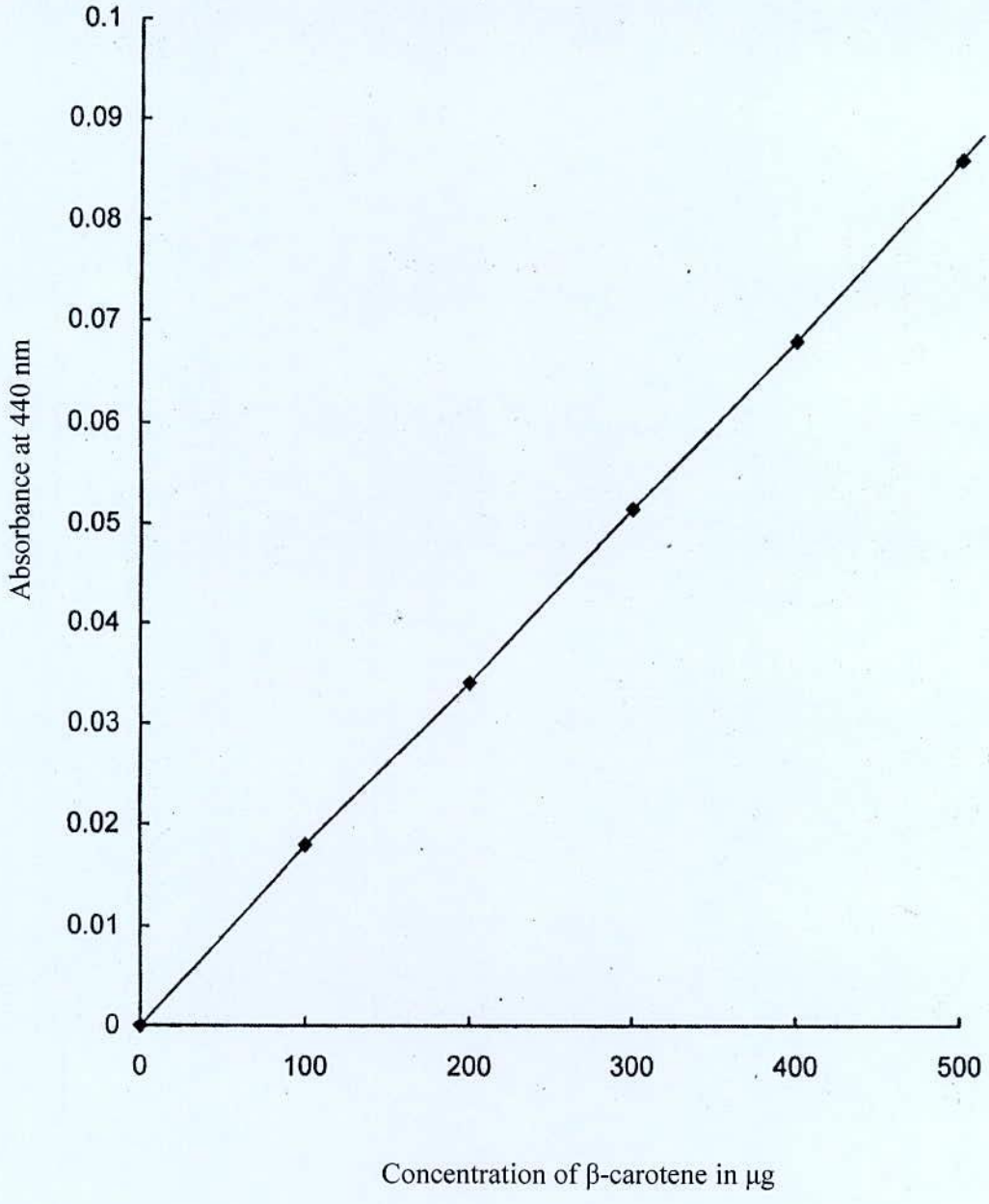


Figure 3.3: Standard curve of β-carotene.

3.2.10 Determination of total protein

Protein content of the treated and untreated mango pulp was determined by the method of Micro-Kjeldahl [66].

Reagents and equipments

a) Solid potassium sulphate
b) Concentrated sulfuric acid
c) 5% $CuSO_4$, $5H_2O$ in distilled water
d) 0.10N H_2SO_4 solution
e) Concentrated sodium hydroxide solution (5 N approximately)
f) Few quartz chips
g) Boric acid solution containing bromocresol green (receiving fluid): 10 g of boric acid was dissolved in hot water (about 250 mL) and cooled. 1 mL of 0.1% bromocresol green in alcohol was added and made upto 500 mL with distilled water.
h) Nitrogen determination apparatus according to Paranas-Wagner, made of JENA Glass-all connections with inter changeable ground joints.

(a) Digestion : Concentrated H_2SO_4 (6-8 mL), 1.0 g K_2SO_4 one to two drops of 5% $CuSO_4$ solution (catalyst) and some quartz chips were added (to avoid bumping) to 3-5 g of mango pulp in a Kjeldahl flask. The mixture was heated till it had become light green (2-3 hrs).

(b) Collection of ammonia: The digestion was carried out in the steam distillation chamber of the nitrogen determination apparatus. The chamber is designated to act as a micro Kjeldahl flask and can be easily detached when needed. After completion of digestion the steam distillation chamber containing the digested mixture was fitted back to the nitrogen determination apparatus. Boric acid solution (15 mL) in a small flask was placed so that the tip of the condenser outlet dipped below the surface of the boric acid solution. Sufficient amount of concentrated sodium hydroxide solution (Approximately, 30-40 mL) was added to the digest in the chamber to neutralize the amount of acid present. Steam was generated from the steam-generating flask and the sample in the chamber was steam distilled until 20 mL of distillate was collected in the boric acid solution. The

condenser outlet was then rinsed with little distilled water and the receiving flask was removed.

(c) Titrimetric examination of ammonia: The ammonia in the boric acid solution was titrated with 0.01N H_2SO_4 until the solution had been brought back to its original yellow green color. The titration was repeated with a control containing only 15 mL of boric acid solution diluted to approximately the final volume of the titrated sample. The volume of acid required was noted.

The nitrogen content was calculated using the formula given below.

1 mL of 0.01N $H_2SO_4 \equiv 140\mu g$ of nitrogen in NH_3.

Thus from the volume of standard H_2SO_4 used for titration, the amount of nitrogen in sample was calculated. The value multiplied by 6.25 give the approximate protein content of the sample used.

Calculation

Amount of protein in the mango pulp (g per 100 g of mango pulp) .

$$= \frac{\text{Amount of protein obtained}}{\text{Weight of mango pulp}} \times 100$$

3.2.11 Determination of iron

Iron content of mango pulp was determined by converting the iron to ferric form using oxidizing agents like potassium persulphate and treating thereafter with potassium thiocyanate to form the red ferric thiocyanate. The absorbance of the solutions were taken at 510 nm in a Coleman Junior 11 spectrophotometer [67].

Reagents

a) Conc. sulphuric acid
b) Saturated potassium persulphate
c) Potassium thiocyanate solution
d) Standard iron solution

Preparation of ash solution

1-2 g of mango pulp was placed in a weighed porcelain crucible (which was previously cleaned and heated to about 100°C, cooled and weighed). The crucible was placed in a muffle furnace for about 18 hrs at about 550°C. It was then cooled in a desiccator and weighed. To ensure completion of ashing, the crucible was again heated in the muffle furnace for half an hour, cooled and weighed again. This was repeated till two consecutive weights were the same and the ash was almost white in color [68]. The ash was moistened with a small amount of distilled water (0.5–1.0 mL) and then 5 mL of conc. HCl was added to it. The mixture was evaporated to dryness on a boiling water bath. Another 5 mL of conc. HCl was added again to the precipitate and the solution was evaporated to dryness as before. Then 4 mL of conc. HCl and a few mL of distilled water were added to the dry ash and the solution was warmed on a boiling water bath. The warmed solution was then filtered into a 100 mL volumetric flask using Whatman No-41 filter paper. After cooling the volume was made upto 100 mL with distilled water and suitable aliquot was used for the estimation of iron.

Procedure

Three different sets of experiments (Blank, Standard and Sample) were performed for the determination of iron. The following different solutions were taken in different 25 mL volumetric flask. In each of the above volumetric flask , made the volume up to 15 mL with water. After mixing the solution, the absorbance of the pink-red colored solution was measured at 480 nm in a colorimeter. The amount of iron (%) in the mango pulp was calculated by using the formula given below.

Calculation

Amount of iron in the mango pulp (mg per 100 g mango pulp)

$$= \frac{\text{OD of Sample} \times 0.1 \times \text{Total volume of ash solution} \times 100}{\text{OD of standard} \times 5 \times \text{Weight of sample taken for ashing}}$$

CHAPTER IV

Results and Discussion

4.1 Effects of antibiotics on the shelf life of fazli mango

It is seen from the table 4.1 to 4.13 that the shelf life of mango fruits was enhancing in different treatment of antibiotics. The shelf life was longer (21 days) in 20 ppm tetracycline and 20, 40, 50, 60 & 100 ppm amoxicillin treated fruits followed by 10, 20, 30 & 40 ppm co-trimoxazole and 30 & 40 ppm ciprofloxacin 20 ppm, azithromycin 20 ppm cefixime and gemifloxacin treated fruits compared to control (16 days).

The foregoing results clearly indicated the efficacy of antibiotics to prolong the shelf life of mango at 15 days commercially effective with 20 ppm tetracycline, 50 ppm amoxicillin, 20 & 30 ppm co-trimoxazole, 50 ppm cefradine, 20 ppm azithromycin antibiotics treatment but that of control is effective at 12 days.

The physiological loss in weights (PLW) of treated and control mango was determined after every day and the results were recorded in table 3.1 to 3.13. It was found that the physiological loss in weight of all sets of treated fruits were lower than that of control.

The loss in weight increased with increasing of storage period. There was a little weight loss in antibiotics treated mangoes compared to control one. 20 ppm tetracycline treated mango had minimum weight loss (29.34%) compared to control mango (46.20%) at 15th day. And at 21st day 20 ppm Co-trimoxazole treated mango had minimum weight loss (72.07%) compared to control (87.91%). Literature is scantly as to how antibiotics control weight loss in stored mango. However it was reported that the percent weight loss in fruits increases with increasing length of storage period regardless of method of ripening [69].

Table 4.1: Weight of tetracycline treated mango (%) at day by day during storage period cultivar of fazli

Treatments	Remaining weight of mango (%)																			
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day	18th day	19th day	20th day	21st day
10 ppm	100	100	97.94	96.35	95.29	94.1	92.67	86.35	85.00	78.22	77.56	76.49	59.86	27.89	27.49	22.01	16.06	10.05	9.83	5.13	4.99
20 ppm	100	100	97.91	96.95	95.61	94.54	93.37	92.22	91.08	90.29	89.55	88.56	77.84	77.11	70.66	49.04	31.90	21.62	16.14	16.02	15.76
30 ppm	100	100	97.78	96.33	95.22	93.94	92.69	91.50	90.15	81.48	63.68	57.44	49.75	31.35	19.65	8.31	8.41	4.15	3.90	3.82	3.72
40 ppm	100	100	98.09	96.55	95.43	88.65	87.33	86.35	85.42	73.22	58.67	52.64	47.13	30.92	25.15	12.13	11.96	11.76	11.62	11.35	0.00
50 ppm	100	100	97.95	96.34	95.17	93.40	92.49	85.61	84.30	78.19	66.70	65.80	58.83	33.54	27.47	27.17	22.94	10.60	10.47	10.44	5.40
60 ppm	100	100	97.90	96.54	95.26	94.10	92.62	91.53	77.49	85.60	71.45	64.41	63.86	56.72	46.22	39.44	23.69	6.00	5.96	5.87	5.86
70 ppm	100	100	97.69	96.25	95.00	93.84	92.14	91.01	89.94	88.93	76.37	69.20	51.73	21.24	16.34	12.63	12.63	6.68	6.64	6.52	6.47
80 ppm	100	100	97.85	96.57	95.10	94.16	92.29	90.97	90.64	90.31	81.87	69.85	40.80	29.80	17.59	11.43	11.50	11.11	11.01	10.57	0.00
90 ppm	100	100	97.76	96.29	95.00	93.97	92.12	90.88	83.57	82.84	63.17	57.29	51.11	16.06	16.00	11.30	11.38	6.14	6.04	6.04	0.00
100 ppm	100	100	97.95	96.47	95.26	93.69	92.50	91.42	90.14	84.15	81.80	69.11	43.96	30.43	30.29	23.55	9.22	4.59	4.47	4.66	4.60

Table 4.2: Weight of amoxicillin treated mango (%) at day by day during storage period cultivar of fazli

Treatments	Remaining weight of mango (%)																				
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day	18th day	19th day	20th day	21st day
10 ppm	100	100	97.93	96.28	95.02	93.55	92.36	91.35	90.42	89.14	82.72	82.01	59.84	53.53	53.07	40.26	23.22	9.90	9.81	5.40	5.39
20 ppm	100	100	98.10	96.50	95.26	94.00	92.62	91.57	90.94	90.85	81.73	63.19	58.63	46.18	40.23	28.17	22.46	17.13	17.08	16.87	10.33
30 ppm	100	100	97.95	96.37	95.01	93.45	92.24	91.11	89.94	88.98	78.41	57.29	45.78	45.08	39.72	39.27	20.60	4.69	4.65	4.43	7.03
40 ppm	100	100	98.23	96.71	95.42	94.00	92.61	91.50	91.20	90.78	76.49	63.54	58.32	47.15	40.13	39.75	26.13	25.67	18.00	11.13	11.20
50 ppm	100	100	97.86	96.29	94.96	93.70	92.06	91.09	90.06	83.65	75.17	73.83	67.57	66.77	60.73	46.82	36.23	30.57	24.70	18.86	18.60
60 ppm	100	100	98.07	96.41	95.12	93.95	92.59	90.97	89.79	88.79	88.13	64.79	59.29	53.92	38.34	32.34	23.04	17.21	17.05	10.68	10.41
70 ppm	100	100	98.06	96.46	95.25	93.81	92.52	86.38	78.64	77.97	71.43	64.29	52.07	29.31	27.70	21.73	21.46	21.21	15.35	9.68	9.52
80 ppm	100	100	97.92	96.23	94.94	94.01	92.05	90.89	84.46	76.52	70.24	64.86	56.50	51.06	38.86	26.40	17.33	11.14	11.07	6.58	6.54
90 ppm	100	100	98.22	96.51	95.21	94.07	92.12	85.04	83.70	82.95	82.19	71.27	70.07	57.23	39.73	21.44	11.67	11.52	11.42	10.68	0.00
100 ppm	100	100	98.04	96.54	95.27	93.93	92.41	91.11	89.72	82.96	82.36	75.85	57.33	46.07	34.84	34.70	29.36	17.53	11.35	11.28	11.11

Table 4.3: Weight of co-trimoxazole treated mango (%) at day by day during storage period cultivar of fazli

Treatments	Remaining weight of mango (%)																			
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day	18th day	19th day	20th day	21st day
10 ppm	100	100	97.81	96.16	94.97	93.48	84.50	83.48	82.32	81.46	80.80	79.56	67.62	66.82	47.67	37.52	27.60	16.12	15.96	15.87	15.58
20 ppm	100	100	97.98	96.41	95.34	93.95	92.44	91.13	89.67	89.33	88.66	87.36	86.40	84.21	69.02	68.29	55.39	38.30	28.69	28.21	27.93
30 ppm	100	100	97.71	96.25	95.04	93.65	91.99	90.76	89.64	88.77	88.02	80.34	73.25	72.49	56.89	50.89	39.01	21.50	21.40	21.30	20.95
40 ppm	100	100	97.82	96.23	94.99	93.59	92.09	90.71	89.25	88.42	87.80	75.69	57.80	44.55	34.36	27.26	16.23	15.89	11.77	11.70	11.63
50 ppm	100	100	97.42	96.13	94.97	93.50	92.02	90.80	89.42	87.19	77.14	69.94	62.21	43.24	27.98	14.05	13.89	8.17	8.10	3.58	3.55

Table 4.4: Weight of ciprofloxacin treated mango (%) at day by day during storage period cultivar of fazli

Treatments	Remaining weight of mango (%)																			
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day	18th day	19th day	20th day	21st day
10 ppm	100	100	97.75	96.27	94.94	93.57	92.00	90.58	82.58	68.52	68.05	57.81	51.42	41.29	25.96	20.90	14.19	7.30	7.25	7.17	7.10
20 ppm	100	100	97.72	96.85	95.22	94.27	92.50	91.23	83.39	82.44	81.71	74.42	63.93	57.57	39.17	38.74	27.46	15.98	9.56	9.21	9.15
30 ppm	100	100	97.95	96.73	95.20	93.77	92.22	91.08	89.69	84.72	83.97	78.01	59.31	52.25	40.44	28.50	17.50	17.36	17.15	16.94	16.57
40 ppm	100	100	97.88	96.60	95.16	93.87	92.35	91.21	89.81	85.47	73.96	73.00	67.00	46.48	35.13	29.25	22.99	16.70	10.67	10.58	10.36
50 ppm	100	100	99.12	97.59	96.21	94.83	93.42	91.88	90.28	85.71	83.56	82.44	51.61	28.81	23.49	23.18	23.01	22.47	9.30	9.25	0.00

Table 4.5: Weight of cefradin treated mango (%) at day by day during storage period cultivar of fazli

Treatments	Remaining weight of mango (%)																			
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day	18th day	19th day	20th day	21st day
10 ppm	100	100	97.84	96.45	89.23	88.00	86.66	85.48	84.00	70.64	70.11	62.26	51.58	40.80	34.71	17.44	11.80	5.68	5.65	5.52	5.57
20 ppm	100	100	98.06	96.63	95.22	93.80	92.43	91.20	84.93	84.69	82.83	75.15	40.22	33.96	24.18	17.67	12.22	5.77	5.74	5.49	0.00
30 ppm	100	100	97.79	96.51	95.17	93.65	92.28	91.34	89.87	88.93	81.88	74.09	63.91	50.32	39.06	33.83	21.53	4.54	4.48	4.46	4.31
40 ppm	100	100	97.55	96.29	94.61	93.30	91.90	90.50	89.11	88.30	82.78	81.71	70.06	57.39	44.16	32.54	20.57	9.28	9.20	4.04	4.01
50 ppm	100	100	97.43	98.55	94.56	93.34	91.68	90.28	88.95	86.84	87.11	81.12	74.20	66.38	65.67	29.63	20.28	9.45	9.37	4.79	4.77

Table 4.6: Weight of azithromycin treated mango (%) at day by day during storage period cultivar of fazli

Treatments	Remaining weight of mango (%)																			
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day	18th day	19th day	20th day	21st day
10 ppm	100	100	97.93	96.68	94.98	93.91	92.07	90.75	89.21	88.61	87.83	81.07	61.86	34.50	29.28	22.76	16.29	11.33	11.24	5.40	5.40
20 ppm	100	100	97.95	96.73	95.16	93.83	92.27	91.21	89.86	82.82	81.90	80.97	69.02	68.31	56.74	44.46	22.02	17.98	17.59	17.30	17.14
30 ppm	100	100	98.02	96.67	95.13	93.51	92.12	90.93	89.54	81.95	80.00	74.40	61.66	55.25	50.24	40.14	20.87	16.01	9.98	9.95	9.71

Table 4.7: Weight of cefixime treated mango (%) at day by day during storage period cultivar of fazli

Treatments	Remaining weight of mango (%)																				
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day	18th day	19th day	20th day	21st day
10 ppm	100	100	97.99	96.73	95.16	93.95	92.37	91.33	83.96	83.20	82.32	72.22	61.02	49.39	35,68	24.01	16.34	12.00	11.78	6.33	6.19
20 ppm	100	100	98.09	96.81	95.21	93.54	92.28	91.16	89.66	77.57	70.52	69.73	64.20	50.87	38.98	33.96	23.70	17.74	10.54	10.47	10.24
30 ppm	100	100	98.48	97.30	96.03	94.43	93.42	92.59	91.81	90.30	74.64	73.76	72.96	53.67	38.95	38.56	23.368	23.25	16.41	4.67	4.58

Table 4.8: Weight of gemifloxacin treated mango (%) at day by day during storage period cultivar of fazli

Treatments	Remaining weight of mango (%)																				
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day	18th day	19th day	20th day	21st day
10 ppm	100	100	98.68	97.45	96.17	94.92	93.54	92.35	91.32	90.37	84.63	78.51	72.69	41.62	34.10	28.43	17.72	11.32	11.11	10.71	13.13
20 ppm	100	100	98.25	97.16	95.79	94.83	93.06	91.80	90.58	89.71	88.95	87.73	53.12	44.70	44.66	38.02	26.87	13.76	9.80	9.56	0.00
30 ppm	100	100	98.38	97.18	95.81	94.72	93.18	86.09	85.08	84.25	83.61	76.35	65.80	49.11	48.79	42.70	30.09	13.28	13.02	8.17	8.02

Table 4.9: Weight of control mango (%) at day by day during storage period cultivar of fazli

Treatments	Remaining weight of mango (%)															
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day
Lot-63	100	100	98.19	96.71	95.45	93.89	92.75	91.47	90.26	89.39	81.80	74.22	61.11	55.10	36.82	31.73
Lot-64	100	100	98.37	97.03	88.71	87.28	86.03	84.93	83.65	82.83	83.38	75.23	67.19	62.16	55.58	37.85

4.2 Effects of antibiotics on general quality

General physical quality of control and antibiotic treated mango were compared by the judges on the basis of appearance, colour, flavor, taste and texture. It can be concluded from their suggestions that the antibiotic treated mangoes are quite superior to that of control one (Table 4.10).

Table 4.10: The grading of control and antibiotic treated mango as judged by the panel of judges based on general qualities of mango

Sample	Treatments	Marking (%)	Order of rating
Appearance	Treated*	95	Excellent
	Control**	75	Good
Colour	Treated	90	Excellent
	Control	70	Fair
Flavour	Treated	88	Excellent
	Control	72	Fair
Taste	Treated	92	Excellent
	Control	80	Good
Texture	Treated	95	Excellent
	Control	78	Good

* Treated: Dipped in solution of antibiotics

** Control: The control were marked and kept at room temperature

4.3 Effects of antibiotics on physiological loss in weight

Physiological loss in weight of control and antibiotic treated mango were compared. It can be concluded from the shelf life study that the antibiotics treated mangoes showed reduced weight loss to that of control one at different concentrations (Fig.4.1-4.12).

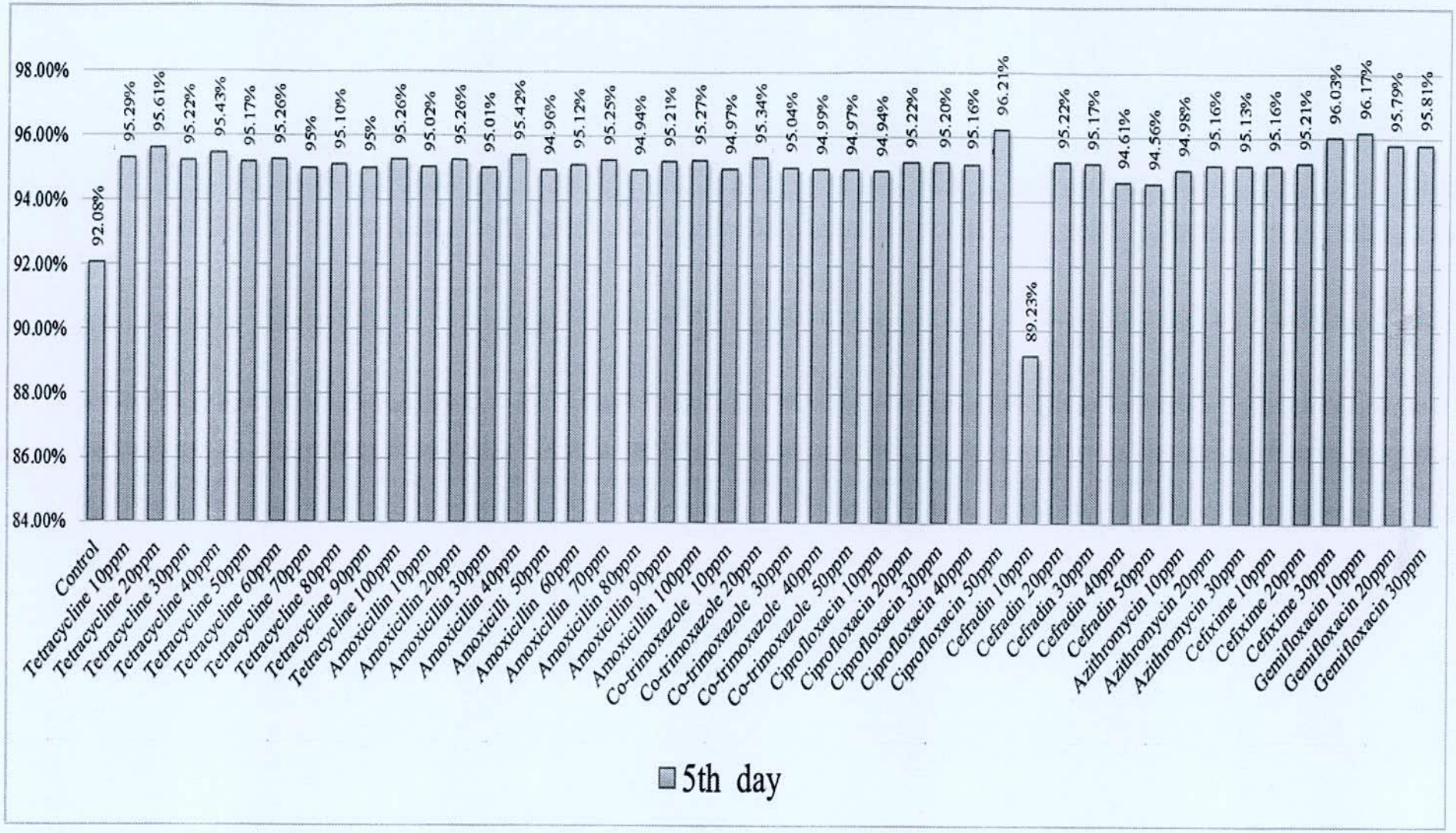


Figure 4.1: Weight of control and antibiotic treated mango at 5th day.

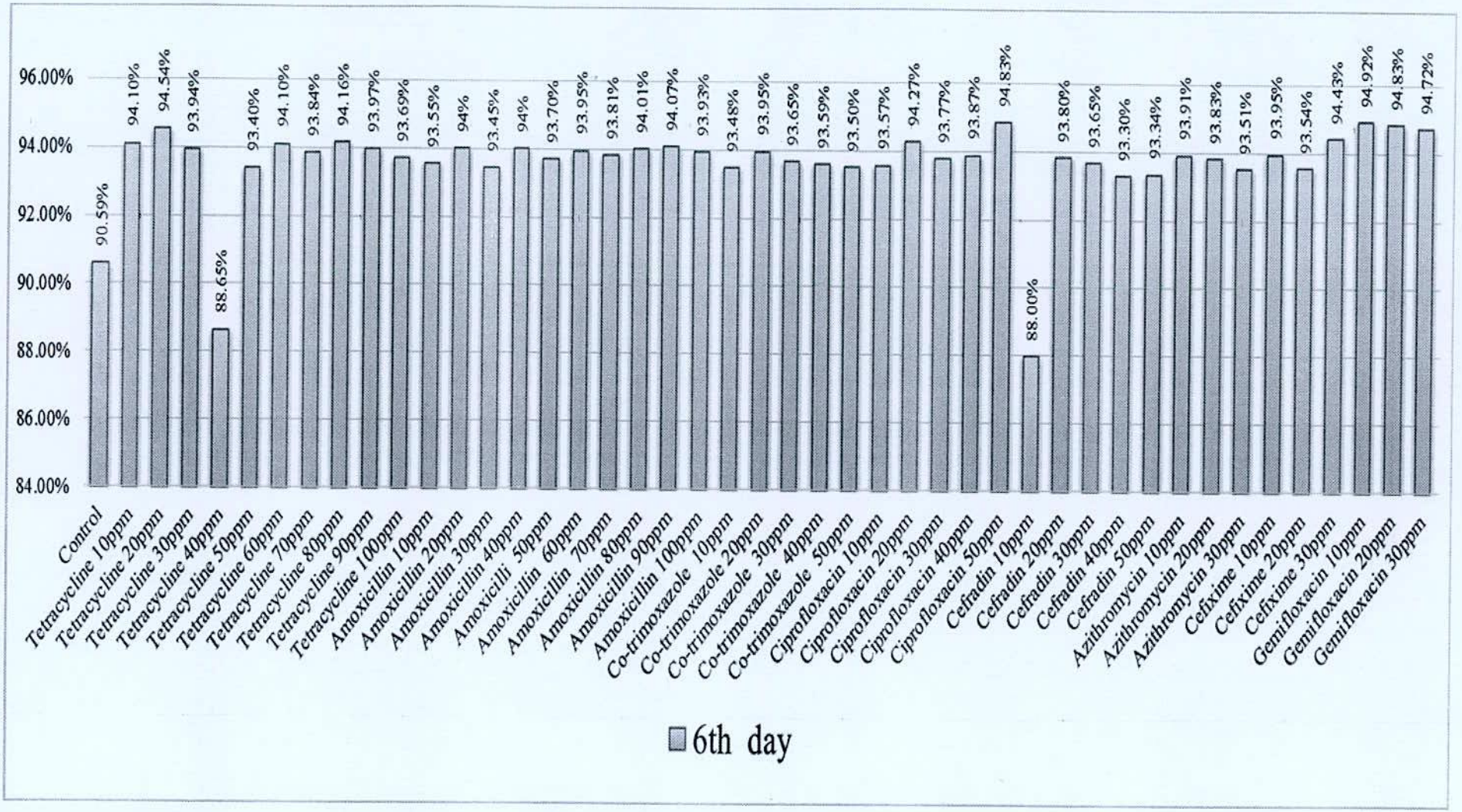


Figure 4.2: Weight of control and antibiotic treated mango at 6th day.

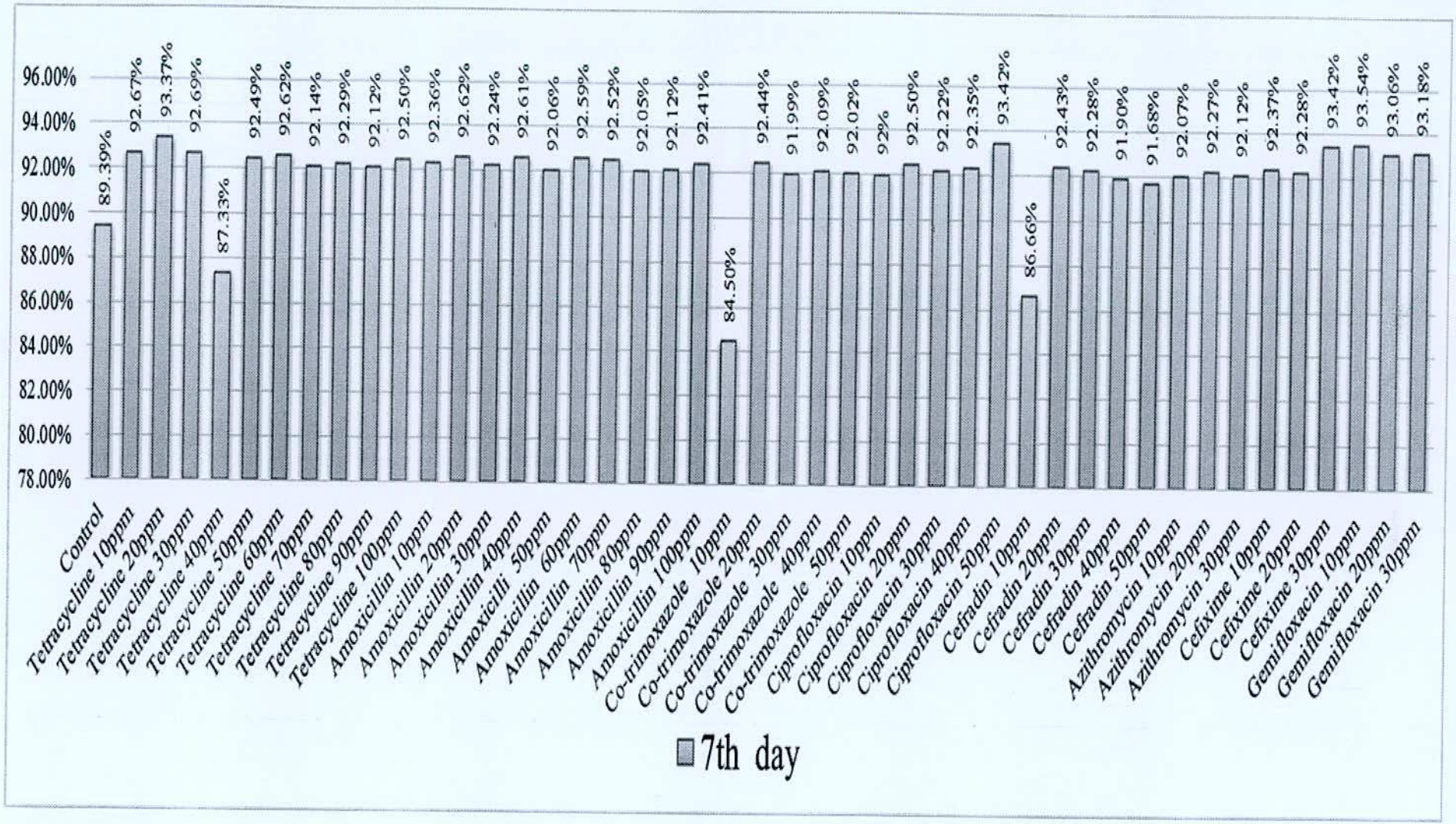


Figure 4.3: Weight of control and antibiotic treated mango at 7th day.

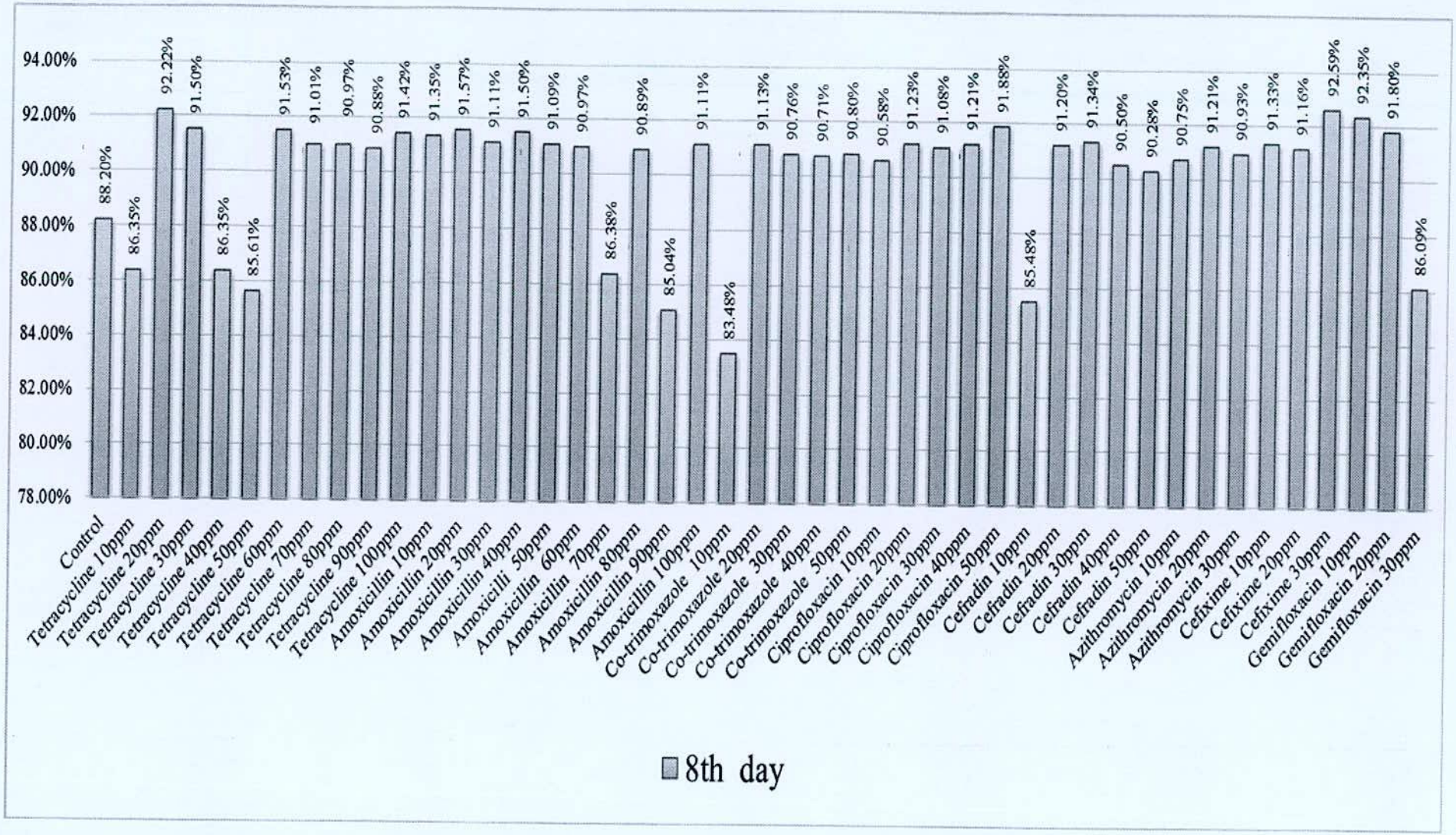


Figure 4.4: Weight of control and antibiotic treated mango at 8th day.

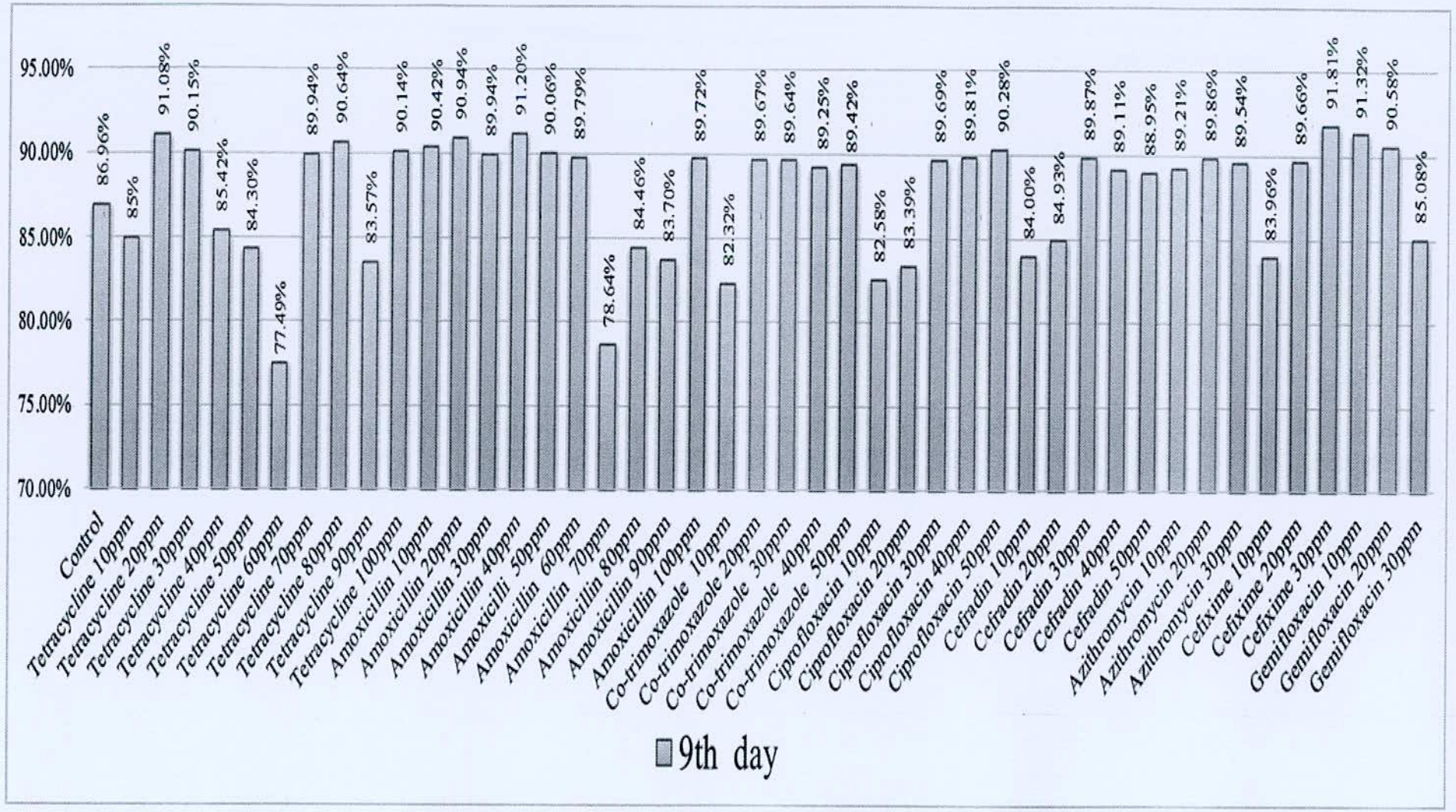


Figure 4.5: Weight of control and antibiotic treated mango at 9th day.

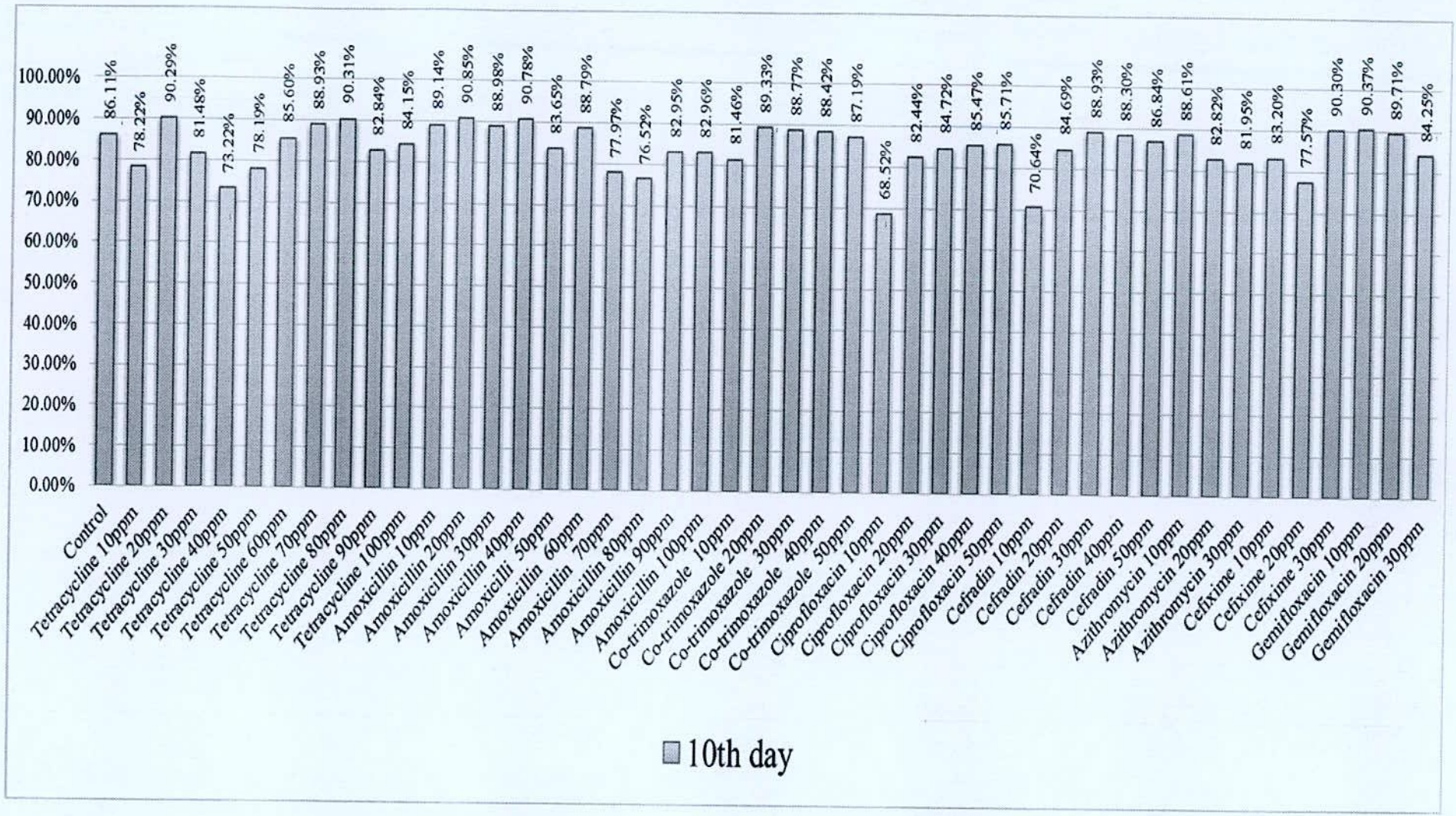


Figure 4.6: Weight of control and antibiotic treated mango at 10th day.

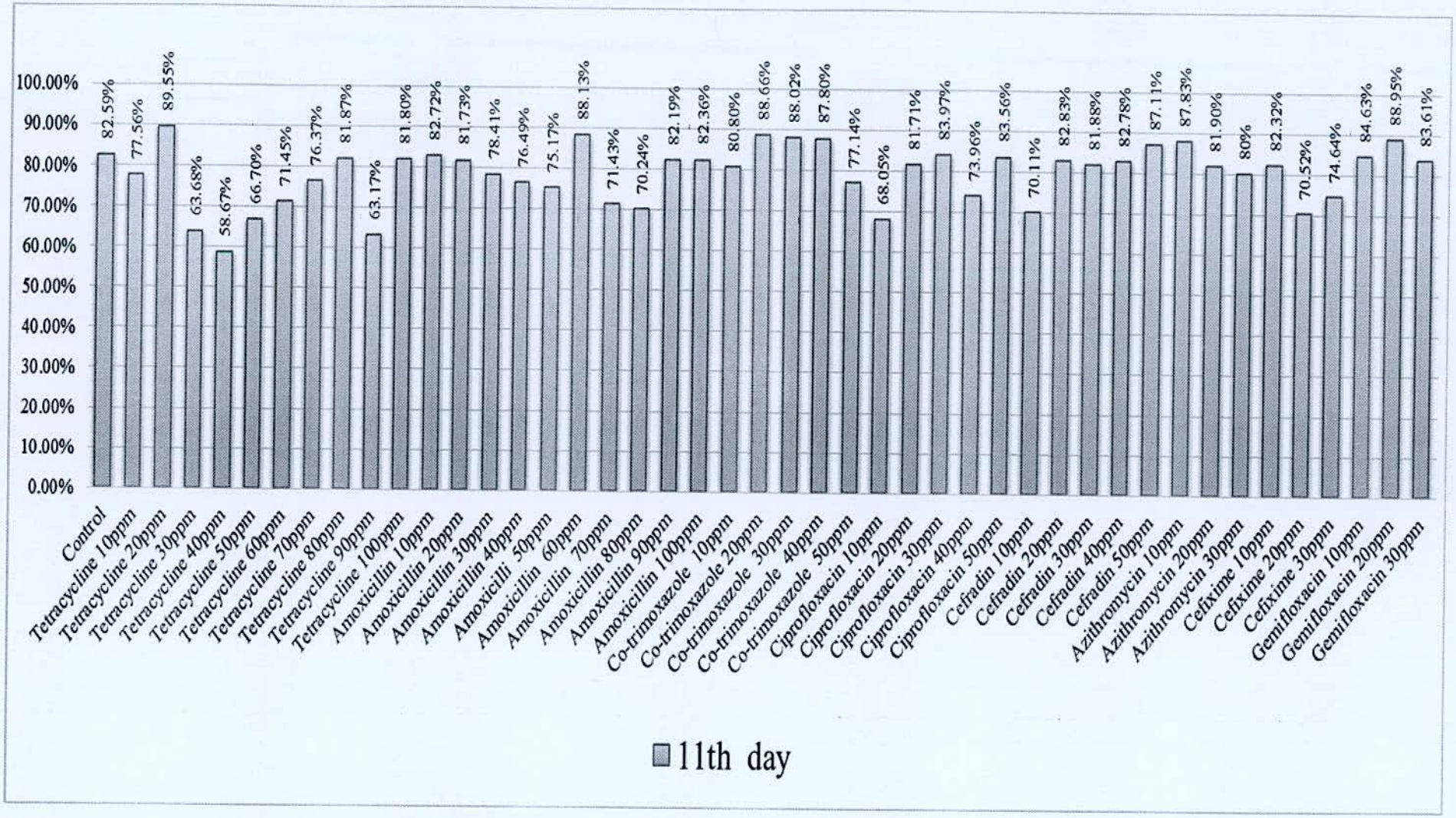


Figure 4.7: Weight of control and antibiotic treated mango at 11th day.

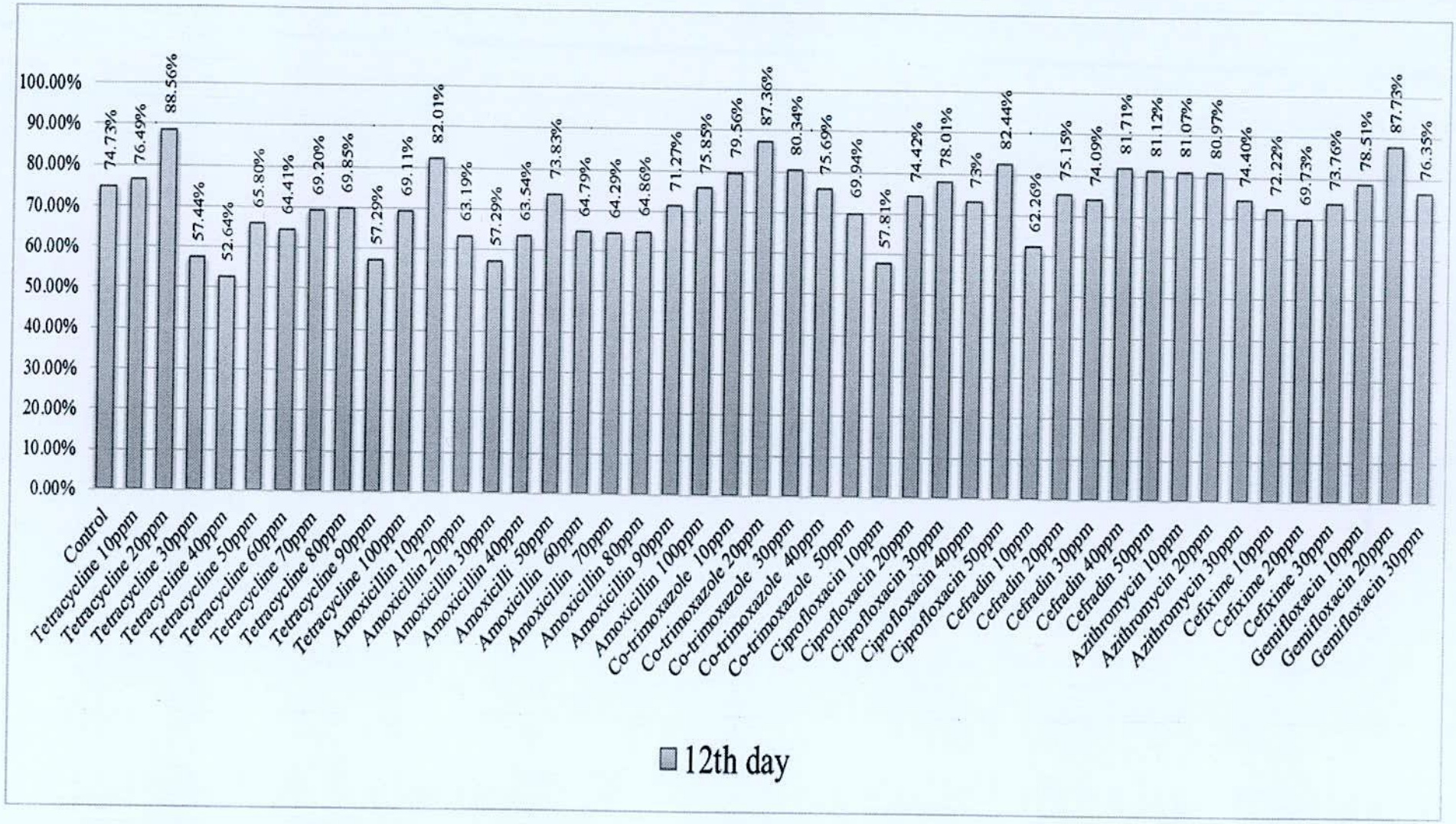


Figure 4.8: Weight of control and antibiotic treated mango at 12th day.

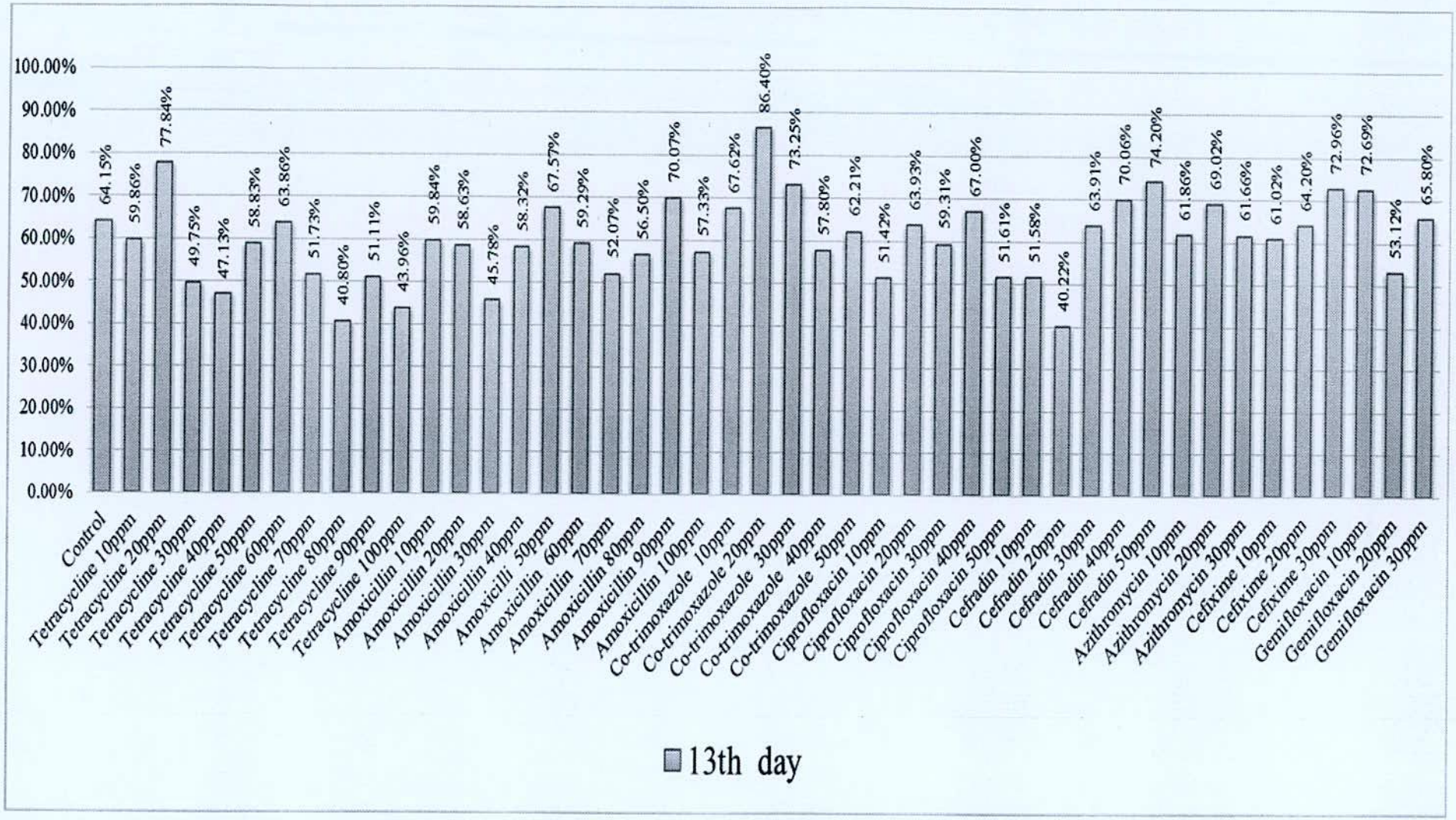


Figure 4.9: Weight of control and antibiotic treated mango at 13th day.

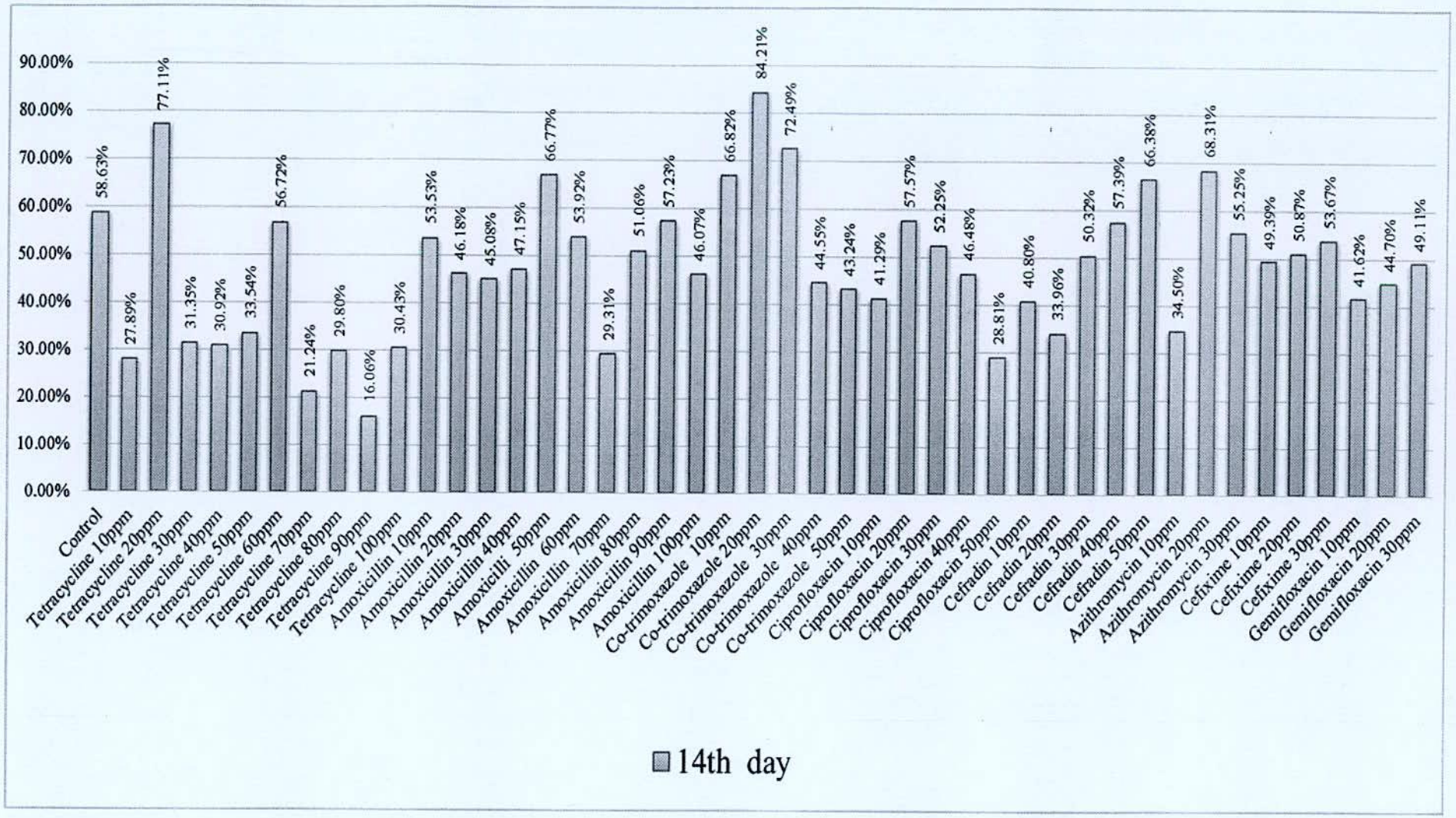


Figure 4.10: Weight of control and antibiotic treated mango at 14th day.

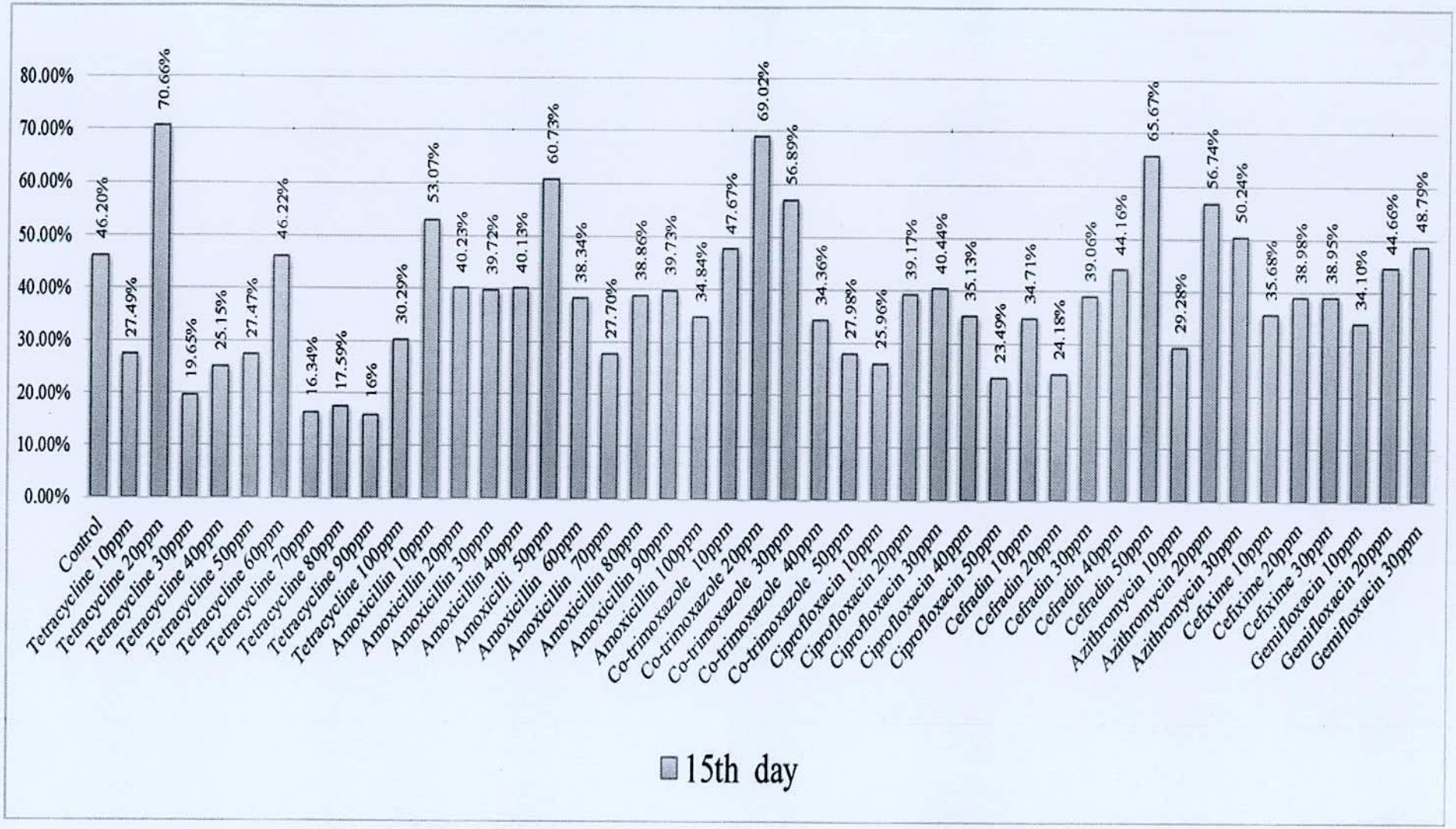


Figure 4.11: Weight of control and antibiotic treated mango at 15th day.

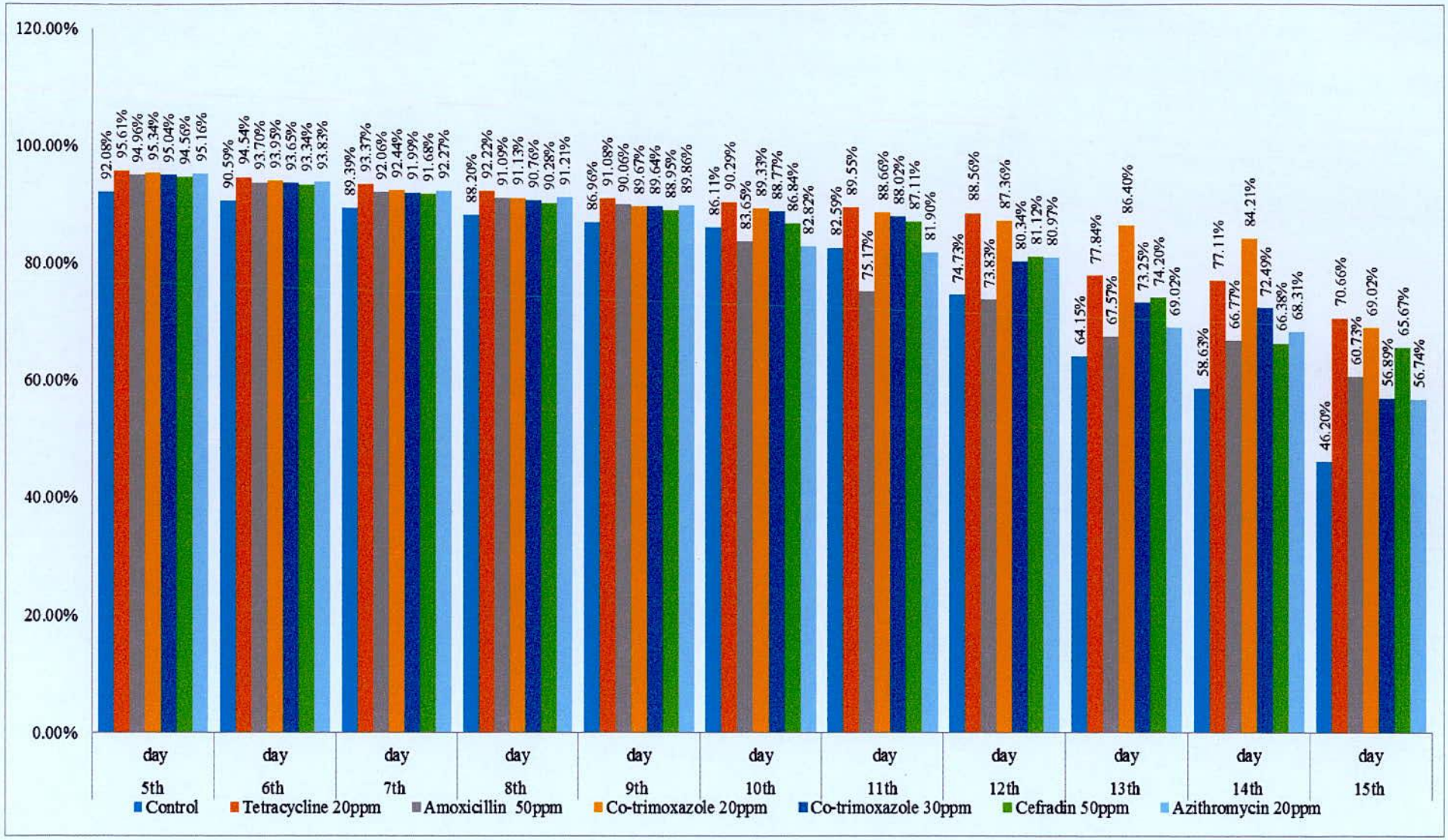


Figure 4.12: Comparative weight of control and antibiotics treated mango at 5th to 15th day.

4.4 Effect of antibiotics on physical appearance

The physical appearance of antibiotic treated mango and control mango were compared. It was found from the physical appearance that the antibiotic treated mangoes showed more attractive appearance to that of control one at the same day in Fig 4.13.1 and 4.13.2.

Figure 4.13.1: The physical appearance of antibiotic treated mango and control mango at 12th day (a) Tetracycline 20 ppm (b) Amoxicillin 10 ppm (c) Amoxicillin 50 ppm (d) Co-trimoxazole 20 ppm (e) Co-trimoxazole 30 ppm (f) Cefradine 50 ppm.

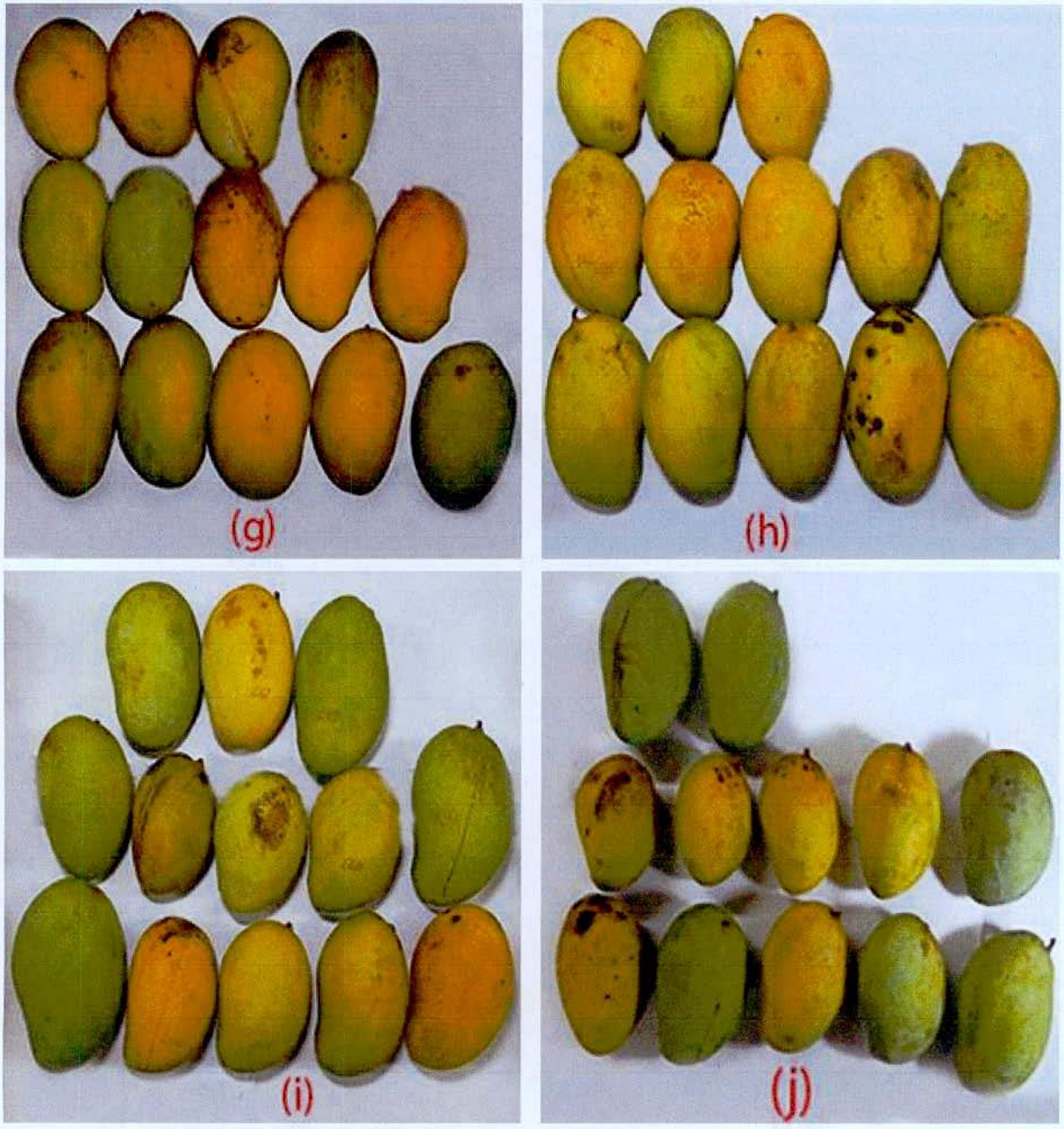


Figure 4.13.2: The physical appearance of antibiotic treated mango and control mango at 12th day (g) Azithromycin 20 ppm (h) Azithromycin 30 ppm (i) Gemifloxacin 30 ppm (j) Control mango.

4.5 Effects of antibiotics on the improvement of quality

pH of mango pulp: As given in table 4.11 the pH in mango pulp was found to be higher in antibiotics treated mango pulp than those in control mango pulp. But at the last edible stage the pH was found to be varied between 5.24 to 6.32 in antibiotics treated mango pulp while that was found to be 5.19 in control mango pulp. The increase of pH was also reported in sweet orange cultivar of Jaffa by Chattopadhyay [70].

Total soluble solids (TSS) of mango pulp: It was found that the TSS was higher in antibiotics treated mango pulp than those in control mango pulp (table 4.11). At the last edible stage the TSS content varied between 11.5% to 19% in antibiotics treated mango pulp while that was found to be 10% in control mango. The increase of TSS was also reported in sweet orange cultivar of Jaffa by Chattopadhyay [70].

Moisture content of mango pulp : As given in Table 4.11, the moisture content in mango pulp was found to be higher in antibiotics treated mango pulp than that of control mango pulp. At ripen stage the moisture content of mango pulp from antibiotics treated mango was found to be varied between 84-87% while that was found to be about 82.66% in control pulp. Similar trends in changes of moisture content were reported in amine mango by Kennard and Winters [71].

Acidity of mango pulp: As given in table 4.11 the acidity in mango pulp was decreased the amount of acidity percentage as citric acid was found to be varied between 0.036 % to 0.07 % as citric acid in antibiotics treated mango pulp while that was found to be 0.08% as citric acid in control mango pulp. Reduction of acidities were also reported in sweet orange cultivar of Jaffa by Chattopadhyay [70], in tomato fruits by Parthasarathy [72].

Ascorbic acid (vitamin C) content of mango pulp: As presented in table 4.11 like acidity, the ascorbic acid content of mango pulp was found to be higher in antibiotics treated mango pulp than those in control mango pulp. At the last edible stage the ascorbic acid was found to be varied between 58.75 mg/100 g to 94 mg/100 g in antibiotics treated mango pulp while that was found to be 47.6 mg/100 g in control. The increase of ascorbic acid was also reported in sweet orange cultivar of Jaffa by Chattopadhyay [70], in goose berry fruits by Gupta V K and Mukherjee D [73].

β-Carotene content of mango pulp: It can be concluded from the data presented in table 4.11 that the carotene was found to be higher in antibiotics treated mango pulp than those in control mango. At the last edible stage the carotene was found to be varied between

7970.4µg/100 g to 7975.3µg/100 g in antibiotic treated mango pulp while that was found to be 5963.2 µg/100 g in control mango pulp. These results are in conformity with those of Hossain and Fattah [74].

Protein content of mango pulp: As presented in table 4.11 like acidity, the protein content of mango pulp was found to be higher in antibiotics treated mango pulp than those in control mango. At the last edible stage the protein was found to be varied between 0.60% to 1.03% in antibiotics treated mango pulp while that was found to be 0.57% in control mango pulp.

Total sugar content of mango pulp: As presented in table 4.11 like pH & TSS, the total sugar content of mango pulp was found to be higher in antibiotics treated mango pulp than those in control mango pulp. At the last edible stage the total sugar was found to be varied between 11.53% to 12.39% in antibiotics treated mango pulp while that was found to be 10.90% in control mango. The increase of total sugar was also reported in sweet orange cultivar of Jaffa by Chattopadhyay [70], in gosse Berry fruits by Gupta V K and Mukherjee D [73].

Reducing sugar content of mango pulp: As given in table 4.11 like pH & TSS, the reducing sugar content of mango pulp was also increased in antibiotics treated mango pulp. At the last edible stage the reducing sugar was found to be varied between 4.73% to 5.46% in antibiotics treated mango pulp while that was found to be 4.60% in control mango pulp. The increase of reducing sugar was also reported in sweet orange cultivar of Jaffa by Chattopadhyay [70], in goose berry fruits by Gupta V K and Mukherjee D [73].

Non-reducing sugar content of mango pulp: As given in table 4.11 like pH & TSS, the higher non-reducing sugar content of mango pulp was found in antibiotics treated mango pulp than those in control pulp. At the last edible stage the non-reducing sugar was found to be varied between 6.50% to 8.00% in antibiotics treated mango pulp while that was found to be 6.30% in control mango. The increase of non-reducing sugar was also reported in sweet orange cultivar of Jaffa by Chattopadhyay [70], in goose Berry fruits by Gupta V K and Mukherjee D [73].

Table 4.11: Comparative physico-chemical data of control and antibiotics treated mango at the last edible stage

Treatments	TSS	pH	Acidity (As citric acid)	Moisture	Vitamin C (mg/100g)	β-Carotene (µg/100g)	Protein %	Fe (Iron) mg/100g	Total Sugar g/100g	Reducing Sugar g/100g	Non-reducing Sugar g/100g
Control	10.0	5.19	0.08	82.66	47.60	5963.2	0.57	0.7218	10.90	4.60	6.30
Tetracycline 20 ppm	12.0	5.25	0.07	87.08	71.10	7972.4	0.79	0.9344	9.79	5.46	4.33
Amoxicillin 50 ppm	11.5	5.25	0.05	86.08	47.00	7975.3	1.03	1.0529	6.59	4.75	1.84
Co-trimoxazole 20 ppm	15.0	6.20	0.05	84.17	94.00	7971.5	0.38	0.4602	8.96	4.69	4.27
Co-trimoxazole 30 ppm	19.0	6.25	0.05	80.23	60.00	7970.4	0.54	0.6204	9.77	4.73	5.04
Ciprofloxacin 20 ppm	18.5	6.15	0.06	81.68	47.00	7973.4	0.49	0.4010	11.53	5.04	6.50
Cefradin 30 ppm	17.0	6.32	0.04	81.38	72.00	7971.3	0.39	0.7858	9.99	4.59	5.40
Azithromycin 20 ppm	15.0	5.65	0.07	82.71	47.60	7972.5	0.46	1.2985	12.39	4.41	8.00
Cefixime 20 ppm	14.0	6.31	0.05	84.49	58.75	7971.0	0.60	0.6909	9.80	4.06	5.74
Cefixime 30 ppm	13.5	5.24	0.07	86.16	35.25	7975.2	0.50	0.7572	10.32	3.95	6.39

CHAPTER V

Conclusion and Recommendations

Even though mango is a delicious juicy fruit produced abundantly in our country but very limited research attention was given to improve the physical and chemical characteristics of such by the application of antibiotics at the post harvest period. Most of the research work has been concentrated in connection of the morphological behavior, variety development and some limited works done on the physico-chemical composition of mango. This research protocol has been undertaken with an objective to control the post harvest losses and to improve the physical as well as chemical characteristics and also to increase the shelf life of mango by the application of antibiotics.

The present study clearly demonstrated that the application of antibiotics solution was very much effective in reducing the physiological loss in weight of mango fruits *in vivo* as well as *in vitro*. So, mango producers might take proper measure after harvesting of mango to control the post harvest losses and also to increase the shelf life of mango by the application of antibiotics. The present data clearly indicated that the physical as well as chemical characteristics of mango were improved significantly with the treatment of antibiotics. Firstly, the shelf life of mango was increased after application of antibiotics. Further the antibiotics treated mango might be considered superior over the control one in respect to the following characteristics, such as development of physical appearance, colour, flavour, taste, texture etc. The antibiotics treated mangoes are also considered to be of good quality in respect to the following characteristics, such as increase of total sugar content, increase of vitamins content, minerals etc. Further the pH of the antibiotics treated mango pulp became slightly higher than control one suggesting the increase of sweetness of preserved mango which also indirectly indicates the improvement of quality of mango by application of antibiotics.

It was also clearly evident from the present study that most of the antibiotics treated mango possessed increase of shelf life and reduction of physiological loss in weight as well as increase of total sugar, total soluble solids and vitamins per 100 g of mango pulp.

In conclusion, the relevant experimental basis has been recommended to the mango growers, wholesalers & the retailers to use the tetracycline 20 ppm, amoxicillin 10 & 50 ppm,

co-trimoxazole 20 & 30 ppm, cefradine 50 ppm, azithromycin 20 & 30 and gemifloxacin 30 ppm solutions as these are the most effective concentrations for the reduction of post harvest losses, extension of shelf life as well as quality of fazli mango.

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